COLD ECOSYSTEMS IN A WARMER WORLD: TRACING RADIOCARBON IN PLANTS AND SOILS OF HIGH ALTITUDES AT DIFFERENT AIR AND SOIL TEMPERATURES

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I dedicate this thesis to my dear cousin Annelise.

"I've seen and met angels wearing the disguise of ordinary people living ordinary lives"

- Tracy Chapman -

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Summary

Summary

Earth's surface mean temperature is rising, and concern about positive feedbacks to warming with it. The alteration of the ecosystem's carbon (C) balance could increase net CO_2 efflux in the atmosphere, and thus further enhance global warming.

Ecosystems from the cold regions are particularly sensitive to climate change. There is increasing evidence that warming of cold ecosystems has enhanced plant productivity (CO_2 sinks) and soil organic matter decomposition (CO_2 sources) over the past decades. In recent years, a number of studies have addressed whether responses of C fluxes to warming in cold ecosystems will result in a net positive or negative feedback to climate change. The interpretation of these observations and extrapolation to future climatic scenarios are challenging, partly because the temperature responses of the C cycling processes involved are not fully understood.

Carbon isotopes can serve as powerful tools, allowing to trace the pathways of C in the plant-soil system. In my thesis, I performed $^{14}CO_2$ labelling experiments to assess temperature effects on C cycling processes and retention mechanism of dissolved organic carbon (DOC) in soils.

In **Chapter 1**, I investigated separate effects of air and soil temperature on C cycling. In a glasshouse study, carried out over spring and summer 2012 and 2013, I factorially manipulated air and soil temperatures (4 or 9 °C) of microcosms containing either *Leucanthemopsis alpina* or *Pinus mugo* seedlings. My results show that soil temperature drives C assimilation and partitioning among plant organs, as well as C transfer to soil via rhizodeposition, while air temperature caused only minor shifts in the belowground distribution of assimilates and rhizodeposits. The respiration of new assimilates in the roots and rhizosphere was more temperature-sensitive than total soil respiration, supporting the hypothesis that cold limitation of C uptake is primarily mediated by reduced sink-strength in the roots. These results indicate that soil temperatures are much more important than air temperatures in driving C cycling response to

warming in cold environments. This is of particular importance given that air and soil temperatures are going to be affected differently by global warming.

In **Chapter 2**, I tested whether soil temperature effects also occur under more natural conditions. I cooled or warmed soil by approx. 6°C in a field experiment near the alpine tree line (Stillberg GR, 2280m) and identified some non-linear temperature relations. C assimilation increased steadily with soil temperature, while assimilates allocation to microbial biomass, fungal hyphae and soil organic matter was particularly low in the cooling treatment, but did not differ between control and warming. The same pattern was also found for the respiration of assimilates in the roots and rhizosphere, confirming that sink-strength is limited by low soil temperatures. I conclude that warming can alter C partitioning between plant biomass and soil pools (root exudates, soil microbial C) and the effect will depend on the initial conditions under which warming occurs.

In **Chapter 3**, retention mechanisms of DOC in soils are addressed. We traced plant derived DO^{14}C across soil columns representing chronosequence of soil development and analyzed DOC retention over the soil profile by ^{14}C autoradiographic imaging. ^{14}C activity in mineralized and leached DOC was also quantified. We observed a strong DOC retention in the topsoil, which correlated with its content of pedogenic oxides. Our results indicate that sorption at mineral surfaces was the main mechanism of DOC retention, while preferential flow across macropores caused DOC leaching under high-flow conditions. These mechanisms are highly relevant for the C cycle because mineral sorption reduces DOC bioavailability, thus increasing DOC residence time in soil. Our results suggest that, if warming increases DOC concentrations via higher litter inputs, the retention and stabilization of this additional C in the soil will depend on soil physio-chemical proprieties and water flow.

Summary

Overall, my findings show that soil temperature drives C uptake and partitioning more than air temperatures. Further, warming effects depend on the temperature range considered; C assimilate use in roots and rhizosphere are especially temperature-sensitive at low soil temperatures, which supports the theory that cold soils hinder C uptake by limiting belowground sink-strength.

Zusammenfassung

Mit dem Anstieg der mittleren Oberflächentemperatur der Erde wächst auch die Befürchtung vor selbstverstärkenden Rückkopplungen zwischen Ökosystemen und dem Klimawandel. Die Veränderung des Kohlenstoffhaushaltes von Ökosystemen könnte den Netto-CO_2-Fluss in die Atmosphäre erhöhen und somit die globale Erwärmung weiter verstärken.
Ökosysteme in kalten Regionen sind besonders empfindlich gegenüber Klimaveränderungen. Wie immer mehr Belege zeigen, führte die Erwärmung von kalten Ökosystemen in den letzten Jahrzehnten zu einer erhöhten Pflanzenproduktivität (CO_2-Senke) aber auch zu einem verstärkten Abbau des organischen Bodensubstanz (CO_2-Quelle). Ob und in welchem Masse die Reaktion der CO_2 Flüsse auf eine Erwärmung von kalten Ökosystemen netto zu einer positiven oder negativen Rückkoppelung mit dem Klimawandel führen wird, ist noch unklar. Die Interpretation dieser Beobachtungen und der Extrapolationen von Klimawandelszenarien ist anspruchsvoll, was zum Teil daran liegt, dass die Temperaturabhängigkeit von Prozessen des Kohlenstoffkreislaufs noch nicht vollständig verstanden wird.
Kohlenstoffisotope sind ein wichtiges Werkzeug, die es erlauben, die ie Pfade des Kohlenstoffes im System ‚Pflanze-Boden' zu verfolgen. In meiner Arbeit untersuchte ich mittels $^{14}CO_2$ Markierungsexperimenten, wie sich Boden und Lufttemperatur auf Prozesse des Kohlenstoffkreislaufs in Ökosystemen auswirken, und welche Mechanismen den Verbleib und Tranport von gelöstem organischem Kohlenstoff (dissolved organic carbon, DOC) in Böden steuern.

Im **ersten Kapitel** untersuchte ich den Einfluss von Boden- und Lufttemperatur auf den Kohlenstoffkreislauf getrennt. In einer Gewächshausstudie manipulierte ich faktoriell Luft- und Bodentemperaturen (4 oder 9°C) in Mikrokosmosen, die entweder *Leucanthemopsis alpina* oder *Pinus mugo* Stecklingen enthielten. Meine Ergebnisse zeigen, dass die Bodentemperatur die Kohlenstoffassimilation und –verteilung zwischen den Pflanzenorganen genauso steuert wie den

Transfer von Kohlenstoff in den Boden durch Rhizodeposition. Die Lufttemperatur bewirkte hingegen nur kleine Änderungen in der unterirdischen Allokation der Assimilate und der Rhizodeposition. Die Respiration von neuen Assimilaten in den Wurzeln oder der Rhizosphäre hing stärker von der Temperatur ab als die Respiration vom gesamten Boden (organische Substanz sowie Wurzeln). Die Befunde stützen die Hypothese, dass die Kältelimitierung der Kohlenstoffaufnahme hauptsächlich durch einen verringerten Kohlenstoffverbrauch in den Wurzeln verursacht wird und nicht durch eine direkte (von der Kälte verursachte) Hemmung der Fotosynthese. Diese Ergebnisse deuten an, dass Bodentemperaturen für die Reaktion des Kohlenstoffkreislaufes auf eine Erwärmung in kalten Regionen viel bedeutsamer sind als Lufttemperaturen. Dies ist besonders wichtig, weil Luft- und Bodentemperatur vom Klimawandel unterschiedlich beeinflusst werden.

Im **zweiten Kapitel** untersuchte ich, wie sich Bodentemperaturen auf den Kohlenstoffkreislauf unter natürlicheren Bedingungen auswirken. In einem Feldexperiment in der Nähe der alpinen Baumgrenze (Stillberg GR, 2280m) kühlte oder erwärmte ich den Boden um etwa 6°C. Dabei ergaben sich einige nichtlineare Temperaturbeziehungen. Die Kohlenstoffassimilation stieg stetig mit der Bodentemperatur an, während die Allokation der Assimilate zwischen Bakterienbiomasse, Pilzhyphen und organischer r Substanz im Boden unter Kühlung besonders niedrig war; sich jedoch nicht zwischen Erwärmung und der unbeeinflussten Kontrolle unterschied. Das gleiche Muster fanden wir auch für die Respiration von Assimilaten in den Wurzeln und der Rhizosphäre, was bestätigt, dass die Kohlenstoffsenke durch niedrige Bodentemperaturen limitiert wird. Zusammenfassend lässt sich sagen, dass eine Erwärmung die Kohlenstoffpartitionierung zwischen der pflanzlichen Biomasse und dem Bodenpool (Wurzelexsudate, Bodenmikroben) beeinflusst und, dass dieser Effekt von den Anfangsbedingungen abhängt, unter denen jeweils die Erwärmung stattfindet.

Im **dritten Kapitel** werden Mechanismen des Transports und Verbleibs von DOC im Boden untersucht. Wir verfolgten streubürtiges DO^{14}C in Bodenprofilen, die eine Zeitabfolge der Bodenentwickelung repräsentierten und analysierten den Verbleib von DOC mittels ^{14}C autoradiographischer Bilder. Die ^{14}C Aktivität in mineralisiertem und ausgewaschenem DOC wurde ebenfalls quantifiziert. Wir beobachteten eine starke DOC-Retention im obersten Boden, die mit der Konzentration von pedogenen Oxiden korreliert war. Unsere Ergebnisse legen daher nahe, dass Sorption an mineralischen Oberflächen der Hauptmechanismus der DOC-Retention war, während präferentieller Abfluss durch Makroporen nur bei sehr jungen, grobkörnigen Böden zur DOC Auswaschung führte. Diese Mechanismen sind von grosser Bedeutung für den Kohlenstoffkreislauf, weil die Sorption an mineralische Oberflächen die Bioverfügbarkeit des DOC verringert, und so die Verweilzeit des Kohlenstoffs im Boden erhöht. Unsere Ergebnisse weisen darauf hin, dass bei einem erhöhtem DOC-Eintrag durch Streu (z.B. unter wärmeren Bedingungen) , der Rückhalt und die Stabilisierung des zusätzlichen Kohlenstoffs von den physikalischen und –chemischen Bodeneigenschaften sowie dem Wasserfluss abhängen werden.

Insgesamt zeigen meine Ergebnisse, dass die Bodentemperatur für die Kohlenstoffaufnahme und –partitionierung wichtiger ist als die Lufttemperatur. Zudem bestimmt der untersuchte Temperaturbereich die Auswirkungen einer Erwärmung; die Verwendung von Kohlenstoffassimilaten in den Wurzeln und der Rhizosphäre reagiert vor allem bei niedrigen Temperaturen sensibel auf Temperaturänderungen. Diese Befunde unterstützten die Theorie, nach der die Kohlenstoffaufnahme bei kalten Temperaturen nicht durch eine von der Kälte verursachte Hemmung der Fotosynthese begrenzt wird, sondern durch den geringeren Kohlenstoffverbrauch im Wurzelraum.

General Introduction

"Knowledge speaks but wisdom listens."

- Jimi Hendrix -

Global warming: aspects and drivers

It is getting warmer. Since the beginning of the 20th century, mean global surface temperatures have increased by almost 1°C. Global warming has recently become more pronounced, advancing at rates of 0.12 °C per decade since 1951 (IPCC, 2013). It is general consensus that warming is driven by increasing concentrations of greenhouse gases in the atmosphere. Greenhouse gases alter the Earth's energy budget, causing a positive radiative forcing which warms the planet's surface. Atmospheric carbon dioxide (CO_2) concentrations have risen by 40% since pre-industrial time, primarily driven by combustion of fossil fuels and changes in land use. CO_2 is the major anthropogenic greenhouse gas, accounting for 76% of total greenhouse gas emission in 2010 (IPCC 2014). Global warming will continue over the next centuries with further increases ranging between 1.0 and 3.7 °C before the end of the 21st century (relative to the 1986-2005 reference period), depending on the scenario considered (IPCC , 2013).

Warming is not homogeneous. Considerable variation exists at the local, regional and temporal scale. There is increasing evidence that warming is more pronounced at high altitudes and latitudes (Bradley, Keimig & Diaz 2004; Rebetez & Reinhard 2007; Rangwala & Miller 2012; IPCC 2013; Pepin *et al.* 2015). Cold regions thus are expected to warm more quickly. Elevation- and latitude-dependent warming results from a multitude of climatic drivers such as cloudiness, direct solar radiation, altitude-dependent vapor saturation and albedo feedback caused by snow and vegetation cover.

Warming effects in cold environments differ above and belowground. The coupling of air and soil temperature depends on environmental factors that are, directly or indirectly, affected by climate change. The presence of snowpack in winter insulates the ground maintaining soil temperature relatively constant and generally above the freezing point, while air temperature

follows diurnal variations and may drop several ten degrees below zero (Mellander, Löfvenius & Laudon 2007; Sutinen *et al.* 2009; Maurer & Bowling 2014). Soils have a large heat capacity which increases with moisture, and consequently the coupling of air and soil temperatures is inversely related to the amount of water that the soil stores (Ochsner, Horton & Ren 2001). In forests, canopies shade the soil preventing warming by direct solar radiation. Consequently, soils under forest canopies are generally cooler compared to soils under low-stature vegetation such as grasslands (Körner 1998; Hiltbrunner, Zimmermann & Hagedorn 2013). Global warming changes snow cover, cloudiness, precipitation, evapotranspiration and vegetation composition. Future air and soil temperatures will therefore be differently affected, both in terms of magnitude and temporal variation (Jungqvist *et al.* 2014).

Warming in cold ecosystems: observed effects and potential threats

Ecosystems of cold regions are expected to be particularly sensitive to warming, because in these environments many biogeochemical processes are constrained by low temperatures (Rustad *et al.* 2001; Aerts, Cornelissen & Dorrepaal 2006a). Effects of warming in cold environments are currently becoming more and more evident at various scales.

Aerial images, local observations and warming experiments have shown an increasing dominance of shrub species in the tundra ecosystem as consequence of rising summer temperature and shorter snow cover duration (Tape, Sturm & Racine 2006; Euskirchen *et al.* 2009; Myers-Smith *et al.* 2011; Elmendorf *et al.* 2012). Similarly, forests are expanding towards higher elevations and latitudes, with a concomitant shift of the alpine and artic treelines (Devi *et al.* 2008; Harsch *et al.* 2009; Hagedorn *et al.* 2014). Plant production in cold environments generally increases with warming because of multiple factors, including an extended growing season and enhanced nutrient availability (Wu *et al.* 1990; Shaver, Bret-Harte & Jones 2001; Rustad *et al.* 2001;

Strömgren & Linder 2002; Natali, Schuur & Rubin 2012). However, temperature responses differ among species and functional groups (Walker *et al.* 2006; Hollister & Flaherty 2010; Elmendorf *et al.* 2012; Sistla *et al.* 2013; Dawes *et al.* 2015), leading to changes in community composition and vegetation structure, including increased dominance of shrubs in the pan-Artic landscape (Sturm, Racine & Tape 2001; Stow *et al.* 2004; Tape *et al.* 2006). Warming also accelerates plant phenology, and stimulates photosynthesis and growth (Rustad *et al.* 2001; Walker *et al.* 2006; Aerts, Cornelissen & Dorrepaal 2006b; Hudson, Henry & Cornwell 2011).

Increased plant production and wooden species dominance suggest that warming in cold ecosystems could increase C storage in form of standing biomass (Hudson & Henry 2009; Melillo *et al.* 2011), therefore increasing CO_2 sequestration. On the other hand, warming enhances microbial decomposition and consequently increases soil CO_2 efflux (Kirschbaum 1995; Rustad *et al.* 2001). Because low temperatures constrain microbial activity, soil from cold regions store large amounts of C in form of labile organic matter (Sjogersten, Turner & Mahieu 2003; Leifeld *et al.* 2009; Tarnocai *et al.* 2009). A small rise in temperature could therefore have major effects on SOM decomposition and lead to a massive release of CO_2 in the atmosphere.

The ultimate feedback of warming on global change will not only depend on which between plant productivity and soil organic matter decomposition will show the stronger response. The temperature-sensitivity of a multitude of processes affecting the coupling of productivity and decomposition will determine which process will dominate the ecosystem's C balance (Trumbore 2006; Chapin *et al.* 2009). Warming effects on belowground C allocation and partitioning among root respiration, biomass, exudates and mycorrhizal associations can strongly affect soil CO_2 fluxes. Because these C pools largely differ in their residence time, the permanence of sequestered C in the soil will depend on its partitioning among them (Chapin *et al.* 2009; Bahn *et al.* 2010).

The C cycle in a nut shell

From the moment when an atom of C is fixed by photosynthesis until it is released back to the atmosphere, this C can pass across a multitude of pools, which are going to determine its turnover time. C assimilates serve as energy source to sustain plant metabolism and be consequently quickly released back in the atmosphere in form of plant respiration. Surplus assimilates are stored as starch that used later when energy demand exceeds assimilation (e.g. at night, or in spring before new leaves are set) (Smith 2001; Zeeman, Kossmann & Smith 2010). Assimilates are also invested in growth, i.e. biomass production, thus entering a pool having a longer turnover time. Belowground, assimilates are also transferred to mycorrhizal symbionts or released to the soil as exudates. Root exudates sustain rhizosphere bacteria and other soil organisms (Strickland, Wickings & Bradford 2012), but part is also leached as dissolved organic carbon (DOC) that eventually gets stabilized by ionic interactions on mineral surfaces (Merino, Nannipieri & Matus 2015).

The time for which an atom of C is removed from the atmosphere, depends on the C pools through which it cycles. It has been estimated that about 50% of assimilated C is respired by plants within 24 h (Carbone & Trumbore 2007). Biomass C has a comparably longer turnover time. Leaf litter generally is decomposed within months to years (Harmon *et al.* 2009). Similarly, fine roots, mycorrhizal mycelium and exudates are easily decomposed and turn over quickly (Gill & Jackson 2000; Kuzyakov *et al.* 2003). Coarse wood is much less susceptible to microbial degradation, often remaining in place for the entire lifespan of a tree and being slowly decomposed afterwards (Weedon *et al.* 2009). Once fragmented, organic C consumed by soil microorganism is used to build up microbial biomass, whose necromass and metabolites contribute substantially to soil organic matter (Schmidt *et al.* 2011). Finally, the organic C that ends up in the soil solution can be stabilized at the surface of the mineral soil phase by ionic

interaction or precipitation (Kalbitz *et al.* 2000). DOC stabilization is going to determine the susceptibility to microbial attack and consequently its residence time in the soil before being mineralized and C released back in the atmosphere.

All of these processes above depend on environmental conditions including temperature. The temperature sensitivities of these processes can differ considerably, while their tight interaction makes it difficult to disentangle direct from indirect temperature effects. In the following section, I review temperature effects on the main processes of the ecosystem's C cycle.

Temperature effects on the main C cycling processes

Photosynthesis is temperature sensitive, reaching its optimum between 23 and 30°C in temperate regions (but it is generally lower in cold environments). Studies have demonstrated that plants can adjust their photosynthetic activity at the temperatures prevailing in the growing season by shifting their optimum as such to maximize photosynthetic rates (Medlyn *et al.* 2002; Grace 2002; Yamori, Hikosaka & Way 2014). Freezing at the beginning or end of the growing season can damage the plant photosystem, reducing C assimilation for up to several days (Oquist & Huner 1991). Some studies revealed an association between elevated sky exposure (causing low night temperatures) and photoinhibition in coniferous seedlings (Germino & Smith 1999; Germino, Smith & Resor 2002; Danby & Hik 2007).

Cold conditions can also indirectly affect photosynthesis by reducing the use and transport of assimilates (Pammenter, Loreto & Sharkey 1993; Iglesias *et al.* 2002; Turnbull, Murthy & Griffin 2002). Negative feedbacks have been observed when cold conditions limit plant use of assimilates, resulting in rising starch concentrations in leaves (Savitch, Gray & Huner 1997; Domisch *et al.* 2002; Hjelm & Ogren 2003; Repo *et al.* 2004). Accordingly, an elevated concentration of non-structural carbohydrates in plant tissues has frequently been observed under

cold conditions (Domisch, Finer & Lehto 2001; Hoch, Popp & Körner 2002; Kontunen-Soppela *et al.* 2002; Hoch & Körner 2009). Such negative feedback on photosynthesis is common in cold environments, because plant growth is more temperature-sensitive that photosynthesis, especially at low temperatures (Grace 2002; Hoch & Körner 2009).

Root growth and wood formation indeed show a non-linear temperature-response and are strongly suppressed when temperature drops below 6-7 °C (Vapaavuori, Rikala & Ryyppo 1992; Alvarez-Uria & Körner 2007; Rossi *et al.* 2007). Although the minimal temperature for root elongation may vary with the natural distribution range of the species (Schenker *et al.* 2014), the pronounced growth suppression below 6-7°C indicates a thermal threshold.

While the temperature dependency of photosynthesis and plant growth are relatively simple, the temperature responses of belowground C fluxes are highly complex and depend on both the direct effect of temperature on plant productivity and the response of root and rhizosphere processes, including root metabolism and mycorrhizal growth. Belowground C flux is tightly coupled to plant production (Litton, Raich & Ryan 2007). Thus, if plant productivity increases under warming, belowground C allocation may increase proportionally. However, because SOM decomposition is as well enhanced by warming, higher nutrient availability can decrease the need for the plant to allocate assimilates to the organs responsible for nutrient absorption (roots and mycorrhiza), therefore changing the proportions between above- and belowground C partitioning (Vogel *et al.* 2008). Studies along natural temperature transects support this mechanism. The proportion of C allocated belowground is negatively related to plant productivity (Litton *et al.* 2007) and decreases with mean annual temperature (Vogel *et al.* 2008; Litton & Giardina 2008; Kane & Vogel 2009). Similarly, an increase in aboveground C partitioning under warming was

observed in experimental studies (Todorovic *et al.* 1999; Hoch 2013; Paradis, Mercier & Boudreau 2014).

If warming alters C partitioning in favor of aboveground organs, C inputs to the soil might increase via aboveground litter fall. Consequently, soil C may not necessarily decrease when SOM decomposition is accelerated because these C losses may be compensated by increased inputs of new plant C (Sistla *et al.* 2013).

Part of the C allocated belowground is consumed by rhizosphere microorganisms, including mycorrhizal fungi and bacteria feeding on root exudates. Because these organisms have a fast turnover, C allocated to these pools will have a short residence time. To date, only few studies were carried out to try separating direct temperature effects on mycorrhizal growth form indirect effects mediated by the response of the host plant (Heinemeyer & Fitter 2004; Gavito *et al.* 2005; Heinemeyer *et al.* 2006; Hawkes *et al.* 2008). These studies revealed a positive relation between soil temperature and hyphal growth, which in all cases was independent of plant biomass and photosynthesis, suggesting that mycorrhizal growth is directly stimulated by temperature. The application of isotope tracing also revealed that quantity and speed of assimilate transfer to the mycorrhizal hyphae increases with temperature (Gavito *et al.* 2005; Heinemeyer *et al.* 2006; Hawkes *et al.* 2008). The fact that the transport of recently fixed C reflects the size of the extraradical hyphal network, and that mycelial activity increases with temperature, suggest that C transfer from the plant to the mycelium is driven by sink strength (sink-regulation).

Fungal and bacterial growth appears less temperature-limited than root growth under cold conditions. The incubation of a large number of mycorrhizal and heterotrophic fungi from temperate to boreal environments for their freezing and low-temperature tolerance (Moser 1958) revealed that strains from high elevation or regularly exposed to low temperatures grew well at

0°C and almost all tested samples grew at 5°C. Likewise, the incubation of forest soils showed that bacterial and fungal growth still occurs at 0°C (Pietikäinen, Pettersson & Bååth 2005). Given that mycorrhiza and rhizosphere bacteria are less temperature-sensitive than root growth, we could expect belowground allocation and respiration of assimilates to continue below temperatures limiting root-growth if they are supplied with substrate.

Finally, under aerobic conditions, soil organic C is respired back in the atmosphere as CO_2. Soil respiration is commonly divided into an autotrophic and heterotrophic part according to the origin of the C respired. These two components are known to exhibit different temperature-sensitivities. Belowground assimilate supply is the main driver of autotrophic soil respiration, considered as the ensemble of root, rhizosphere and mycorrhizal respiration (Lin, Rygiewicz & Ehleringer 2001; Heinemeyer *et al.* 2007; Moyano, Kutsch & Rebmann 2008; Fenn, Malhi & Morecroft 2010; Subke *et al.* 2011). The relative contribution of autotrophic respiration to total soil CO_2 efflux depends on vegetation type (Hanson *et al.* 2000) and varies with season, being highest during the summer (Epron *et al.* 2001; Schindlbacher, Zechmeister-Boltenstern & Jandl 2009), when it makes up to 60% of total soil CO_2 efflux in boreal and temperate forests (Högberg *et al.* 2001; Subke, Hahn & Battipaglia 2004; Moyano *et al.* 2008). It is argued that autotrophic respiration is less or not affected by temperature, because it highly dependent on plant photosynthetic activity and assimilates transfer (Atkin, Edwards & Loveys 2000; Hartley *et al.* 2007; Subke *et al.* 2011; Wang *et al.* 2014). The increase in soil CO_2 efflux observed under warming is therefore mainly driven by soil organic matter decomposition and mineralization, rather than by root, mycorrhizal and rhizosphere respiration. Changes in belowground allocation of plant assimilates could however alter the contribution of autotropic respiration to the total soil CO_2 efflux.

The CO_2 efflux generated by SOM decomposition and mineralization constitutes the heterotrophic component of soil respiration. The temperature sensitivity of SOM decomposition is not constant, but varies with the temperature range considered, being much more responsive at 0°C than at 20°C (Kirschbaum 1995). Heterotrophic respiration also depends on many other environmental variables and is not affected by warming if moisture (Rustad & Fernandez 1998), substrate diffusion (Zak *et al.* 1999), or microbial access to C pools (MacDonald, Zak & Pregitzer 1995) are limiting.

In a number of warming experiments, the response of soil CO_2 efflux to warming decreases over time (Luo *et al.* 2001; Melillo *et al.* 2002; Strömgren & Linder 2002; Niinistö, Silvola & Kellomäki 2004). This phenomenon of acclimation has been associated with thermal adaptation of soil microbial communities (Luo *et al.* 2001; Bradford *et al.* 2008) and substrate depletion of labile C pools resulting from accelerated rates of SOM mineralization (Knorr *et al.* 2005; Eliasson *et al.* 2005). Although the most labile C fraction can deplete quickly, some authors warned that the decomposition of recalcitrant C pools could be even more temperature-sensitive than labile C pools because the decomposition of recalcitrant components requires more activation energy (Bol *et al.* 2003; Fierer *et al.* 2005; Knorr *et al.* 2005; Davidson & Janssens 2006). These authors attribute the apparent insensitivity observed to the relative short duration of incubation studies relative to the long turnover time of these pools, which can vary from centuries to millennia (Trumbore 2000). Because of the elevated amount of organic C stored in soils of tundra, boreal and alpine regions, warming effects on soil CO_2 efflux are expected to be stronger in these regions (Davidson & Janssens 2006; Leifeld *et al.* 2009; Hagedorn *et al.* 2010). Furthermore, the ongoing unthawing of permafrost is exposing a growing fraction of soil organic

C to microbial degradation, with potential major feedbacks on global warming (Schuur *et al.* 2009; Tarnocai *et al.* 2009; Natali *et al.* 2011).

Sources and retention mechanisms of DOC

DOC originates from root exudates, plant litter, microbial biomass and solubilized organic matter. It constituted by organic molecules of various sizes and commonly defined as the filtrate obtained by passing soil solution thorough a 45µm pore size (Kalbitz *et al.* 2000). DOC concentration correlates to plant productivity (Fröberg2006) and increases with temperature either because litter production (substrate) is enhanced, or because microbial activity is stimulated and therefore the processes mediating DOC productions as well (Freeman *et al.* 2001; Neff & Hooper 2002; Harrison *et al.* 2008).

DOC retention mechanisms determine whether DOC inputs are biodegraded, stabilized or leached.

Generally DOC concentrations decrease with depth in mineral soils. It is however not clear whether such decrease results from microbial degradation of DOC or sorption at the mineral surface (Müller, Alewell & Hagedorn 2009). While the first mechanisms would imply that DOC has a fast turnover, mineral sorption reduces DOC bioavailability, enhancing its residence time. Preferential flow through soil macropores can as well contribute to DOC removal, especially during storm events when the rapid flow reduces the contact between soil solution and mineral surfaces, thus increasing DOC leaching to the deep soil (Hagedorn *et al.* 2000).

The mechanisms controlling DOC cycling in soil and their interactions are not yet fully understood.

Their investigation is timely and relevant because which prevails is going to determine whether larger DOC inputs under warming will also enhance soil C stocks in the long term.

The application of C isotopes to the tracing of C fluxes

Carbon isotopes can serve as powerful tools, allowing to trace the pathways of C cycling in the different components of the plant-soil system. Labelling with $^{14}CO_2$ and $^{13}CO_2$ followed by tracing of the label in soil CO_2 efflux has often been applied to quantify time lags between C assimilation and release from the soil, as well as mean residence time of assimilates in different plant and soil C pools (Carbone & Trumbore 2007; Högberg *et al.* 2008; Kuzyakov & Gavrichkova 2010). This technique has also been applied to separate soil CO_2 effluxes between roots plus rhizosphere and bulk soil respiration with the advantage of minimizing soil disturbance (Hanson *et al.* 2000). A further advantage of radioisotopes is that the spatial distribution of ^{14}C assimilates can be visualized by phosphor imaging (Finlay & Read 1986; Rosling, Lindahl & Finlay 2004; Pausch & Kuzyakov 2011; Dawes *et al.* 2013).

Reviewing 47 pulse-labelling studies, Epron et al (2012) pointed out that C assimilation and belowground transfer are tightly coupled, with time lags that can vary from few minutes up to few days, depending on the distance between photosynthesizing tissues and sinks. Roots and microbial communities are as well tightly coupled as assimilated C was detected almost simultaneously in roots and microbial biomass. In this review the author also noticed that the assimilate transfer occurs faster in broadleaved than coniferous species, likely reflecting differences in phloem anatomy.

Despite their versatility, C isotopes have been rarely applied to the investigation of temperature effects on C assimilation and partitioning. Results of experiments involving the use of C isotopes suggested that both transport rates (Streit *et al.* 2013; Barthel *et al.* 2014) and aboveground partitioning (Ge *et al.* 2012; Kasurinen *et al.* 2012) of assimilates increase with temperatures in cold environments. More studies are however needed understand the mechanisms behind these observed responses.

Thesis outline

This thesis focuses on the temperature relations of C uptake and partitioning under cold conditions. More specifically, my research aims at disentangling air and soil temperature effects on C cycling processes and investigates the existence of thermal-thresholds and non-linear temperature relations.

I investigate temperature effects on net C uptake, partitioning to roots, microbial biomass, mycorrhizal hyphae, DOC and bulk soil, by conducting $^{14}CO_2$ labelling experiments and tracing the ^{14}C label in the plant and soil system. The temperature-response of respiration derived from recent assimilates (soil $^{14}CO_2$ efflux) and total soil respiration (soil CO_2 efflux) were also compared. I made use of ^{14}C autographic imaging to visualize ^{14}C assimilate distribution in roots and soil. This technique was also applied to visualize the small scale retention of ^{14}C labelled DOC within soils columns.

I have decided to work at rather low temperatures, to understand how temperature affects plant and soil processes in cold environments, which are expected to be particularly sensitive to warming. To understand the relative role of air and soil temperatures and their interactions, I manipulated these two components separately, investigating which of the two has the stronger effect on the observed responses.

In **Chapter 1**, I describe a glasshouse study in which I factorially manipulated air and soil temperatures under controlled conditions, aiming at understanding which of these temperatures have the stronger effect on net C uptake, assimilate partitioning and respiration.

In **Chapter 2**, I moved towards more natural conditions and performed a temperature manipulation experiment in the Swiss Alps near the treeline over summer 2012. This study focused on the effect of soil temperature, by warming or cooling the soil above and below natural conditions occurring during the growing season. By having three equally spaced temperature

levels, I could test whether temperature responses of uptake, partitioning and respiration of ^{14}C are linear or some threshold relations exist.

Chapter 3 describes A DO^{14}C tracer experiment carried out by Nadia Bruderhofer during her master thesis at the ETHZ.

In her project, we traced the leaching of plant derived DO^{14}C across soil columns representing sequential stages of soil development, investigating the mechanisms responsible for DOC retention in soils. I have contributed to this chapter by supervising the quantification of ^{14}C activity in soil samples and the preparation of soil cores for autoradiographic imaging. I have furthermore quantified, visualized and statistically analyzed DO^{14}C distributions in the autoradiographic images and contributed to the writing of the paper that resulted from our work.

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Chapter 1

Interactive effects of air and soil temperature on C allocation in cold-adapted plant species: A ^{14}C pulse labelling experiment

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Abstract

Ecosystems of cold regions likely are particularly sensitive to global warming because many processes are temperature-limited. Predictions of global warming effects on C cycling in cold ecosystems are difficult due to the close coupling of above- and belowground processes. Further, global warming will affect air and soil temperatures differently.

We factorially manipulated air and soil temperatures (4 or 9 °C) of microcosms containing either *Leucanthemopsis alpina* or *Pinus mugo* seedlings. After seven days, these microcosms were pulse-labelled with $^{14}CO_2$. For four days after labelling, soil CO_2 and $^{14}CO_2$ evolution was quantified. Then, the microcosms were destructively harvested and ^{14}C quantified in plant and soil fractions.

Plant biomass, net ^{14}C uptake and transfer to soil in *L. alpina* were, respectively, 25, 42 and 82% higher at 9 than at 4 °C soil temperature. Soil warming also increased the amount (+70%) and depth of ^{14}C allocated to *L. alpina* roots, with no corresponding effects of air temperature. In *P. mugo*, there was an interactive effect of air and soil temperature on ^{14}C partitioning, with the highest fraction of ^{14}C allocated to roots in the 9 °C air/4 °C soil temperature combination. Soil $^{14}CO_2$ efflux increased more with soil temperature than CO_2 efflux (98% and 214% in *L. alpina* and *P. mugo,* respectively).

These findings highlight the dependence of C uptake, use and partitioning on both air and soil temperature, with the latter being relatively more important. The strong temperature-sensitivity of C assimilate use in the roots and rhizosphere supports the hypothesis that cold limitation on C uptake is primarily mediated by reduced sink strength in the roots. We conclude that variations in soil rather than air temperature are going to drive plant responses to warming in cold environment, with potentially large changes in C cycling due to enhanced transfer of plant-derived C to soils.

Introduction

Climate warming is more pronounced at high latitudes and altitudes (Rangwala & Miller 2012; IPCC 2013; Pepin *et al.* 2015). Ecosystem responses to warming may be particularly large in these cold regions because many plant and soil processes are limited by low temperatures (Aerts, Cornelissen & Dorrepaal 2006). Higher temperatures in cold ecosystems generally stimulate plant assimilation (Rustad *et al.* 2001; Wu *et al.* 2011; Myers-Smith *et al.* 2011; Elmendorf *et al.* 2012) However, warming also increases plant respiration (Atkin, Edwards & Loveys 2000; Schindlbacher, Zechmeister-Boltenstern & Jandl 2009) and microbial decomposition in soil (Kirschbaum 1995; Knorr *et al.* 2005; Streit *et al.* 2014). Whether C storage in plant and soil pools will increase or decrease is thus difficult to predict. The reason for our limited understanding of C cycling responses to future conditions (De Deyn, Cornelissen & Bardgett 2008; Chapin *et al.* 2009) is that (1) the different processes tightly interact, even though they (2) are spatially separated.

Soil microbial respiration increases strongly with temperature (Kirschbaum 1995), although it is largely driven by plant C supply when longer time frames are considered (Bahn *et al.* 2006; Caprez, Niklaus & Körner 2012; Heinemeyer *et al.* 2012). Differences also exist between soil fractions, with rhizosphere respiration depending more on plant photosynthetic activity (Moyano, Kutsch & Rebmann 2008; Fenn, Malhi & Morecroft 2010) than bulk soil (Hartley *et al.* 2007; Wang *et al.* 2014). Above and belowground processes further experience different temperatures due to their spatial separation. Soil temperature broadly follows air temperature, but the coupling can be affected by many factors including snow cover (Maurer & Bowling 2014), soil moisture (Ochsner, Horton & Ren 2001), and canopy shade (Körner 1998). Different global change effects on air and soil temperatures are expected (Zhang *et al.* 2005; Jungqvist *et al.* 2014), but warming experiments have rarely been laid out and interpreted with respect to different temperature

changes above and belowground (Pumpanen *et al.* 2012; Hoch 2013). While open-top chambers generally lead to larger air than soil temperature increases (Hobbie & Chapin 1998; Hollister & Webber 2006), heating cables in soil or overhead infra-red lamps predominantly warm soils, with smaller changes of air temperature.

Carbon isotopes offer powerful techniques to study above and belowground C cycling while minimizing disturbance (Hanson *et al.* 2000). Tracer studies have shown that plant assimilates are rapidly transferred belowground and used by soil microbial communities (Kuzyakov & Gavrichkova 2010; Epron *et al.* 2012), with time lags that can vary depending on plant size, phenology and xylem morphology. Only a few studies have applied C isotope tracers to investigate temperature effects on plant C dynamics. The transport of ^{13}C-labelled assimilates to roots in *Larix decidua* was found to be slower at high than at low altitudes, whereas leaf non-structural carbohydrates concentration at high elevation were larger, suggesting a sink limitation of plant growth in cold environments (Streit *et al.* 2013). Similarly, warming increased ^{13}C fixation and biomass partitioning to foliage relative to non-photosynthetic tissues in *Betula pendula* (Kasurinen *et al.* 2012) and in the boreal grass *Phalarsis arundinacea* (Ge *et al.* 2012). Finally, little is known on temperature effects on rhizosphere C dynamics.

Our study focuses on the interactive effects of air and soil temperatures, aiming at disentangling their effects on C allocation in cold-adapted species. Two plant species commonly found at the alpine treeline were exposed to a factorial manipulation of above and belowground temperatures. Pulse-labelling with $^{14}CO_2$ was used to trace the fate of recent assimilates through the plant and soil system. We quantified ^{14}C in above and belowground pools (microbial biomass, fungal hyphae, and bulk soil). We also trapped soil respiration and applied an autoradiographic technique to map the small-scale belowground distribution of ^{14}C assimilates (Pausch & Kuzyakov 2011; Stiehl-Braun *et al.* 2011; Hagedorn *et al.* 2015). Specifically, we were interested

in whether above or belowground temperatures exert stronger controls on C cycling, and whether these two effects interact.

Materials and Methods

Experimental design

We factorially manipulated air and soil temperatures in a greenhouse study with microcosms containing seedlings of either *Leucanthemopsis alpina* (L., Heywood) or *Pinus mugo* (Turra). The applied temperature levels were 4 and 9 °C, with diurnal amplitudes of approximately 6 °C for air and 2 °C for soils.

The air temperature treatment was applied in a 120 × 54 × 52 cm (length × width × height) gas-tight acrylic chamber with two fan-equipped radiators placed at both ends of the chamber through which 50% aqueous ethylene glycol was circulated from a 200 L cooling tank (Pro-Inox CV-G 200, Galactea, Cleppe, France, 1 °C day and -2.5 °C night temperature). Air temperature was fine-tuned manually by periodically adjusting the coolant flow with tube clamps (Fig. 1). We had only one acrylic chamber available, and therefore repeated the experiment serially, randomly assigning 4 and 9 °C air temperature treatments to sequential pairs of runs. In total, there were four runs with *L. alpina* (2012, April 30th–July 7th) and eight runs with *P. mugo* (2013, February 4th–June 1st), i.e. there were two and four replicates for each air temperature.

The acrylic chamber contained eight heating/cooling aluminum blocks allowing to separately manipulate soil temperature of 8 microcosms. The bottom 2.8 cm of each microcosm was tightly fit into an aluminum block through which a coolant was circulated. Soils of microcosms in the 4°C soil treatment were cooled using the coolant that controlled air temperature, whereas an independent water bath set at higher temperature was used in the 9 °C soil treatment. Soil

temperature (10.7 cm depth) was continuously adjusted to target levels (Fig. 1) by pulsed electrical heating (100W heating element, custom-built electronics controlled by LabView, National Instruments, Austin, TX). Two pairs of microcosms were located in each half of the acrylic chamber. We randomly assigned 4 and 9 °C soil temperature treatments to microcosm pairs, separately for each chamber half and run.

Plant material and microcosm set up

All plants originated from seeds collected in the Swiss Alps (2000-2200 m a.s.l.). Three-week old *L. alpina* seedlings were provided by Schutz-Filisur Alpin Gartencenter (Filisur, Switzerland). Two-year old *P. mugo* saplings were grown in WSL's experimental garden (Birmensdorf, Switzerland). In October 2011, plants were potted in sieved (7 mm mesh) top soil collected at Stillberg in the Swiss Alps (2200 m a.s.l., see Hagedorn et al 2008 for details), in microcosms constructed from 10 cm inner diameter × 15 cm length polyethylene tube sections (Geberit AG, Jona, Switzerland) closed at the bottom with a 2 mm nylon mesh. A 3 cm diameter × 12 cm length 50 μm nylon mesh bag filled with 80 g carbon-free quartz sand was placed vertically in the soil to assess fungal hyphae ingrowth (Wallander *et al.* 2001). The microcosms with seedlings were kept for 7 (*L. alpina*) and 16 months (*P. mugo*) in the greenhouse prior to treatment application (day and night-time temperatures of 12-14 °C and 8-10 °C, respectively, 15 hours photoperiod with additional illumination when ambient light dropped below 40 klx).

Soil moisture was adjusted to 60% water holding capacity and the microcosms insulated with a 1 cm thick tubular insulation foam before being placed into the acrylic chamber where they were exposed to target temperatures for 7 days. During this time, plants were watered three times with 20 ml each. The acrylic chamber was left slightly open to maintain CO_2 concentration around ambient concentrations.

^{14}C pulse-labelling

The chamber was sealed and a $^{14}CO_2$ pulse released at once by acidification of $Na_2{}^{14}CO_3$ solution in a glass bulb through which chamber air was circulated. The chamber was kept closed for a further 24 hours during which headspace CO_2 concentration was maintained between 300 and 500 ppm (LI-6200, Licor, Lincoln, NE) by releasing CO_2 from unlabeled Na_2CO_3. The amount of ^{14}C released varied with run; for *L. alpina*, an equivalent of 57.5 kBq ^{14}C per microcosm was released (note, however, that the amount of label was controlled at the chamber and not the microcosm level). For *P. mugo*, an equivalent 100 kBq ^{14}C per microcosm were released but this amount was raised to 650 kBq when microcosms dedicated to autoradiographic analyses were in the chamber. Twenty-four hours after the initial label release, the chamber was opened and plants kept at target temperatures for a further four days during which soil solution and soil CO_2 efflux were collected. We also added water to compensate moisture losses estimated in the previous weeks.

Dissolved organic carbon (DOC)

Soil solution was collected using 10 cm long samplers (Rhizon CSS, Rhizosphere, Wageningen, Netherlands) inserted horizontally into the plant rooting zone at 5 and 10 cm soil depth. Soil solution was extracted by attaching pre-evacuated 12 ml vials to the samplers. The vials were replaced every 24h and the solution frozen until dissolved organic ^{14}C ($DO^{14}C$) quantification by liquid scintillation counting (TriCarb 2900, Packard BioScience, Meriden, CT; 1 ml sample plus 4 ml Ultima Gold cocktail, Perkin Elmer, Waltham, MA).

Soil respiration

Each microcosm was crossed at 5 and 10 cm depth by 11 cm long sections of hydrophobic gas-permeable tubing (Accurel PP V8/2HF, Membrana GmbH, Wuppertal, Germany). Soil air was pumped (IPC-N 24 peristaltic pump, Ismatec, Glattbrugg, Switzerland; 2 ml min^{-1} flow rate) through 35 ml 0.5 M NaOH placed in a 50 ml vial before the now CO_2-free air was circulated back to the hydrophobic tubing. We also trapped soil surface CO_2 efflux in *L. alpina* microcosms but encountered technical difficulties and therefore analyzed total soil respiration trapped in both hydrophobic tubings instead. When labelling *P. mugo*, we estimated soil respiration with a static micro-chamber (2.7 cm diameter × 6 cm length test tube inserted 3 cm into the ground) into which we placed a vial with 2 mL 1M NaOH. Soil respiration trapping started when the chamber was opened (i.e. 24 h after $^{14}CO_2$ release). Earlier trapping would have removed $^{14}CO_2$ from the chamber headspace, thus confounding values of soil $^{14}CO_2$ efflux. All NaOH solutions were replaced every 24 hours and trapped CO_2 quantified by acid titration after CO_3^{2-} precipitation with $BaCl_2$, using phenolphthalein as pH indicator (Alef & Nannipieri 1995). $^{14}CO_2$ was determined by liquid scintillation counting as described above. For *P. mugo*, total CO_2 and $^{14}CO_2$ evolution were scaled up to the microcosm's surface area.

Destructive harvest and analysis of plant and soil material

Four days after labelling, plant shoots were clipped at soil level and dried at 75 °C. Microcosms used for autoradiographic imaging of belowground ^{14}C distribution were immediately frozen and processed as described in the next section. For microcosms used for destructive analyses, the hyphal ingrowth bag was divided into three parts equal in length and frozen. Hyphal ^{14}C was determined later by suspending each section in 250 ml H_2O using a blender and vacuum-filtering a 50 ml aliquot (Whatman 42 filter paper, GE Healthcare, Freiburg, Germany) to collect the

hyphae. The filter was dried (70 °C). Soil and roots were collected separately from 0-5, 5-10 and 5-15 cm depth sections. Roots were washed and dried (75 °C). The root-free soil was sieved (2 mm) and an aliquot stored at 4 °C for microbial biomass determination. Another soil aliquot was dried at 105 °C for bulk ^{14}C analysis. Dry plant material was ground or cut into small pieces (*P. mugo* branches).

^{14}C in plant material, fungal hyphae and soil was quantified by liquid scintillation counting (6 mL Carbosorb E mixed to 12 mL Permafluor E, Perkin Elmer, Waltham, MA) of evolved $^{14}CO_2$ after sample combustion in Packard 307 sample oxidizer (Perkin Elmer, Waltham, MA).

Soil microbial C was determined by chloroform fumigation-extraction (Vance et al 1987). In brief, 10 g soil were extracted with 30 ml 0.05 M Na_2SO_4 (45 min, 150 rpm), filtered (MN 615, Macherey-Nagel AG, Oensingen, Switzerland) and ^{14}C in the filtrate quantified as described for $DO^{14}C$. A second sample was processed similarly after fumigation with ethanol-free chloroform. Carbon concentrations in the extracts were measured with a Dimatoc 2000 TOC analyser (Dimatec Analysentechnik GmbH, Germany). Microbial C and ^{14}C were calculated assuming an extraction efficiency of k_{EC}=0.45 (Wu et al 1990).

For *L. alpina*, ^{14}C in microbial biomass, fungal hyphae, and DOC were below the detection limit, most likely because of the lower amount ^{14}C used during pulse-labelling and are thus, not reported here.

Autoradiography of soil sections

The frozen, intact, belowground part of the microcosms were freeze-dried. Then, microcosms were placed upright in 15 cm diameter Petri dishes with semi-hardened epoxy resin (Laromin C 260, BASF, Ludwigshafen, Germany, mixed at a ratio of 2:3 with Araldite DY 026 SP hardener, Astorit AG, Einsiedeln, Switzerland) to seal the bottom of the polyethylene tubes. Once the resin

had hardened, fresh resin was added from the top and air removed from soil pores by slowly evacuating the cores in a desiccator to -20 kPa and slowly bringing pressure back to atmospheric levels (Stiehl-Braun *et al.* 2011). The resin was left curing at room temperature for three days before it was fully hardened for 24 hours at 60 °C. The tubes were then cut length-wise with a circular saw and removed from the resin core. Using a diamond saw (Discoplan TS, Struers GmbH, Birmensdorf, Switzerland), a 6 mm thick vertical section was separated from the center of each resin core. This section was divided into four rectangular pieces approx. 5 × 7 cm in size that were mounted on glass slides, ground flat with a diamond cup mill, and used to expose phosphor image plates (BAS III S, Fujifilm, Tokyo, Japan; 7 and 10 days exposure for *P. mugo* and *L. alpina* soils). The imaging plates were scanned at a resolution of 200 µm (Fujix BAS 1000 scanner, Fujifilm, Tokyo, Japan) and scans of the four parts recomposed. Background exposure was subtracted from the data and the depth distribution of the recorded activity determined (MATLAB 2012b, MathWorks, Natick, MA). For this analysis, areas containing the highly-labelled main root of *P. mugo* were excluded since their occurrence strongly depended on where the soil core had been cut.

Statistical analyses

All data were analyzed by fitting linear mixed-effects models that reflected the structure of the experimental design (ASReml 3.0, VSNI International, Hempstead, UK). Fixed effects were block, soil and air temperature (coded as two-level factors), and plant species. Blocks refer to replicates in time, i.e. consecutive pairs of runs at low and high air temperature. Random effects were run (the replicate for the air temperature treatment) and chamber halve. The soil temperature treatment was applied to pairs of microcosm; data were aggregated at this level so that pair did

not have to be included as random effect except when multiple measures per microcosm pair were analyzed together (e.g. soil layers or repeated measures).

It should be noted that our design did not allow for tests of air temperature effects on absolute amounts of ^{14}C. The reason is that microcosms exposed to low and high air temperature were labelled separately, with the amount of ^{14}C released in each run largely determining total uptake, so that differences in assimilation rates could not manifest. However, the analysis of the proportional distribution of ^{14}C among ecosystem compartments is unaffected by this caveat. This limitation does not apply to tests of soil temperature effects, since microcosms exposed to low and high soil temperature were exposed to the same atmospheric ^{14}C concentrations.

Results

Plant biomass

Biomass of about 7 month old *L. alpina* was 25% higher at 9 °C soil temperature than at 4 °C ($F_{1,6}$=10.3, P<0.05, Fig. 2). In contrast, biomass of 4-year old *P. mugo* saplings did not respond to any temperature treatment. In both species, root to shoot ratios did not change with temperature.

Microcosm net ^{14}C uptake

About 50% of released ^{14}C was recovered in plant plus soil material four days after labelling. In microcosms planted with *L. alpina*, 41% more ^{14}C was found at 9 °C than at 4 °C soil temperature ($F_{1, 6}$=14.0, P<0.01, Fig.3). No treatment effect on net ^{14}C uptake was found in *P. mugo* microcosms.

^{14}C partitioning

The fraction of plant ^{14}C recovered in *L. alpina* roots increased from 4.7% in cold soil to 8.0% in warm soil ($F_{1,6}$=7.6, P<0.05, Fig. 3). In contrast, in *P. mugo* the fraction of plant ^{14}C in roots depended on the interaction with air temperature ($F_{1,17}$=19.3, P<0.001 for air × soil temperature) and it was lowest when both air and soil were cold (29.3%) and highest when air was warm but soil cold (45.5%).

Soil ^{14}C activity in *L. alpina* microcosms increased by 82% with soil temperature ($F_{1,6}$=11.2, P<0.05, Fig. 3). However, no such effect was found when soil ^{14}C was expressed as fraction of total microcosm ^{14}C, which amounted to 5.4 and 6.7% in cold and warm soils, respectively. No effects of soil temperature on soil ^{14}C were found in *P. mugo*.

Vertical ^{14}C distribution in roots and soils

In general, soil temperature effects on ^{14}C depth distribution were larger in *L. alpina* than in *P. mugo* and more pronounced in the deep soil (Fig. 4). Root ^{14}C in *L. alpina* decreased from 0.62 kBq in the upper to 0.08 kBq in the lower layer in cold soil (Fig. 3), but remained constant in warm soil, averaging 0.64 kBq per layer ($F_{1,14}$=24.3, P<0.001, for depth × soil temperature). The magnitude of the effect increased with soil depth and ^{14}C in the lower layer was 6 times higher at 9 °C than at 4 °C air temperature (Fig. 4). Vertical depth distribution of root ^{14}C and of root biomass differed ($F_{1,74}$=4.1, P<0.05, for depth × variable) and the vertical distribution of *L. alpina* roots was not affected by soil temperature (Fig. 2).

Root ^{14}C in *P. mugo* decreased steeply over the soil profile in all treatment combinations, except when both air and soil temperatures were set at 4 °C ($F_{2,29}$=3.3, P=0.05 for depth × air temperature × soil temperature, Fig. 3). The vertical ^{14}C distribution in *P. mugo* roots did not

follow root biomass ($F_{1,108}$=10.6, P<0.01, for depth × variable, Fig. 2), with the latter not varying among layers.

For both species, soil ^{14}C, i.e. net rhizodeposition, approximately followed root ^{14}C distributions (Fig. 3), with significant soil temperature effects in *L. alpina* ($F_{1,14}$=8.0, P<0.05) but not in *P. mugo* microcosms.

Microbial ^{14}C in *P. mugo* microcosms averaged 60% of total soil ^{14}C (Fig. 3) with no effects of air or soil temperature. Similarly, hyphae ^{14}C showed no temperature effects in *P. mugo* and remained below detection limit in *L. alpina*.

Soil respiration

Both soil CO_2 and $^{14}CO_2$ efflux (Figs 5 and 6) increased with soil temperature ($F_{1,24}$=15.6, P<0.001 and $F_{1,25}$=12.4, P<0.01, for CO_2 and $^{14}CO_2$ respectively). These effects did not depend on air temperature.

For both species, soil temperature effects were larger for $^{14}CO_2$ than for CO_2 efflux ($F_{1,39}$=5.4, P<0.05, for variable × soil temperature). The difference between $^{14}CO_2$ and CO_2 response to temperature was species-specific ($F_{2,38}$=17.7, P<0.001, for species × variable × soil temperature), with $^{14}CO_2$ efflux increasing proportionally more with warming in *P. mugo* microcosms (Fig. 4).

On average, soil CO_2 efflux increased by 54% and 5% with soil temperature in *L. alpina* and *P. mugo,* respectively. Soil respired $^{14}CO_2$ was more temperature-sensitive, increasing by 98% in *L. alpina* and by 214% in *P. mugo*, which corresponds to Q_{10} values of 4.6 and 13.8, respectively (Fig. 4). For *P. mugo,* the ratio of soil-respired $^{14}CO_2$ to net ^{14}C uptake was three times larger in warm than in cold soils ($F_{1,10}$=7.9, P<0.05). For *L. alpina,* this ratio was twice as large in the 4 °C air −9 °C soil combination than when both air and soil temperatures were 4 °C ($F_{1,6}$=7.5, P<0.05).

Soil $^{14}CO_2$ decreased more quickly with time (Fig. 5) in *L. alpina* than in *P. mugo* microcosms ($F_{1,86}$=82.2, P<0.001, Figs. 5), but the decrease was not affected by air or soil temperatures.

Autoradiographic imaging

Total ^{14}C recorded in autoradiographies did not depend on soil temperature in any species. In *P. mugo*, however, ^{14}C was relatively more concentrated in the upper third of the pot when air was cold, while more ^{14}C was allocated into the deeper soil when air was warm (air × soil temperature, $F_{3,28}$=3.78, P<0.05; Fig. 6). Mean ^{14}C allocation depth in *P. mugo* microcosms increased with air and soil temperature ($F_{2,6}$=5.85 , P<0.05) and averaged to 6.0, 6.8, 7.1 and 7.8 cm (for air/soil temperature combinations of 4/4 °C, 9/4 °C, 4/9 °C and 9/9 °C, respectively). No temperature effects were found in *L. alpina*.

Discussion

In *L. alpina,* the positive soil temperature effect on net ^{14}C uptake and plant biomass, combined with the fact that these were independent of air temperature, strongly suggests that the mechanisms controlling net C uptake are located below rather than above ground. The temperature sensitivity of ^{14}C labelled rhizosphere respiration was particularly high, indicating that, for both species studied, low temperature decreased the use of recent assimilates in roots. Most likely, cold soils constrained C uptake via a reduced assimilate sink-strength in roots. Our finding is in line with previous studies that demonstrated the temperature-dependency of root metabolism (Ericsson, Rytter & Vapaavuori 1996; Iivonen & Rikala 1999; Pregitzer *et al.* 2000).

If air temperatures were not strongly limiting, net assimilation would ultimately track belowground sink activity, possibly because non-structural C accumulates in leaves (Domisch, Finer & Lehto 2001; Hoch, Popp & Körner 2002; Kontunen-Soppela *et al.* 2002; Hoch & Körner

2009) and inhibits photosynthesis (Paul & Foyer 2001; Turnbull, Murthy & Griffin 2002). We therefore interpret our findings of a small net ^{14}C uptake at low soil temperature in conjunction with a reduced respiration of recent assimilates as support of the idea that cold limitation of C uptake is primarily mediated by belowground C sinks. This mechanisms has been proposed as primary explanation for the worldwide coincidence of climatic treelines with a distinct growing season soil temperature (Körner 1998).

Root growth is temperature-dependent and strongly repressed below ≈6 °C (Vapaavuori, Rikala & Ryyppo 1992; Alvarez-Uria & Körner 2007; Schenker *et al.* 2014). In our study, we demonstrate that not only growth but also root respiration and rhizodeposition are limited at low temperatures. In the cold soil treatment, ^{14}C contents were lower in roots, soil CO_2 efflux, and soil organic matter, indicating a decreased ^{14}C allocation to roots and reduced rhizosphere metabolic activity. The strong temperature response of soil $^{14}CO_2$ efflux in *P. mugo* suggests that the turnover of new assimilates in the roots is highly temperature-dependent under cold conditions. Temperature responses of soil CO_2 efflux were comparably much weaker. This contrasts observations of higher temperature dependencies of total soil respiration compared to root and rhizosphere respiration (Hartley *et al.* 2007; Hagedorn *et al.* 2010; Streit *et al.* 2014; Vogel *et al.* 2014; Wang *et al.* 2014) which usually are explained by a strong temperature dependency of microbial activity (Hartley *et al.* 2007). In a recent soil warming experiment at the alpine tree line, Ferrari, Hagedorn & Niklaus (2015) have shown that the temperature sensitivity of rhizosphere respiration was higher than that of total soil respiration between 5 and 10 °C soil temperature, but smaller between 10 and 15 °C. This indicates that the relative contribution of rhizosphere to soil CO_2 efflux depends on the temperature range considered, with stronger responses around threshold temperatures limiting root activity.

Soil temperature limitation of root activity is further supported by the vertical distribution of root ^{14}C over the soil profile. For both species, ^{14}C activity tended to decrease towards the bottom of the pot. A larger fraction of ^{14}C activity was recovered in the deep soil at 9 that at 4 °C. The same trend was observed in *P. mugo* autoradiographies, confirming the relation between soil temperature and depth allocation of new assimilates. Overall, this indicates that faster C turnover in the warmer soil induced a larger sink for assimilates.

While the observed responses depended only on soil temperature for *L. alpina*, an interaction between air and soil treatments was found in *P.* mugo. The effect of soil warming on the ^{14}C allocation to *P. mugo* roots tended to decrease with air temperature. Our results indicate an interdependence of C allocation among plant organs, with above ground temperature affecting belowground processes and *vice-versa*. Such an interactive effect is supported by biomass measurements from a reciprocal air and soil temperatures manipulation study performed in growth chambers under similar conditions (Hoch 2013). The observed interaction could result from a combination of above- and belowground limiting processes. In their review of plant phenology observations from 40 different studies, Abramoff and Finzi (2015) hypothesized an endogenous control of above ground phenology on root activity. The specific mechanisms are not yet clear but seems to rely on changes in C assimilate supply and hormonal signaling.

It is difficult to assess whether, in cold ecosystems, the enhancement of plant growth under warming (Rustad *et al.* 2001; Dawes *et al.* 2015) results from a direct temperature effect, or if it is mediated by a concomitant increase in nutrient availability driven by higher mineralization rates (Dormann & Woodin 2002; Melillo *et al.* 2002; Dieleman *et al.* 2012). In our study, larger soil CO_2 efflux suggests a stimulation of organic matter mineralization in the warm soil treatment. A concomitant increase in nutrient availability can therefore not be excluded. Nevertheless, studies having manipulated both temperature and nutrient supply indicated that

warming *per se* can stimulate plant productivity, because an addition of nutrients equivalent to what would be released under warming could not yield the same increase in biomass (King et al 1999; Hoch 2013; Paradis et al 2014). A direct temperature effect on plant growth is further supported by increasing C partitioning to roots when warming is applied under cold (growth-limiting) conditions (Domisch *et al.* 2001; Hawkes *et al.* 2008; Karst & Landhäusser 2013; Virjamo, Sutinen & Julkunen-Tiitto 2014). If higher productivity would be driven by enhanced nutrient availability, then a smaller response of belowground biomass would be expected.

We explored short-term effects of air and soil temperature changes, which likely are transient responses and might differ from longer-term effects. In this study *P. mugo* net ^{14}C uptake did not increase with soil temperature, although previous studies under similar conditions demonstrated a positive relation in alpine coniferous species when soil temperatures were risen over a longer period of time (Hoch 2013; Dawes *et al.* 2015; Ferrari *et al.* 2015). In our study, it is likely that the shorter acclimation period preceding ^{14}C labelling (seven days) was not long enough to drive tangible effects on net C uptake in *P. mugo*, because it may take longer for assimilates to accumulate and inhibit photosynthesis, especially in trees (Zhou & Quebedeaux 2003; Hjelm & Ogren 2003). This could also explain why net ^{14}C uptake increased with temperature in *L. alpina*, but not in *P. mugo*. The proportion of photosynthesizing *vs.* non photosynthesizing plant biomass was much higher in *L. alpina* than in *P. mugo*. Accumulation of new assimilates and the resulting negative feedback on photosynthesis would therefore take longer to develop in *P. mugo* compared to *L. alpina*. Plausibly when plants were ^{14}C labelled, photosynthesis was already inhibited in *L. alpina* in the 4 °C soil treatment, while *P. mugo* plants assigned to the same treatment were still able to accumulate ^{14}C assimilates in form of non-structural carbohydrates.

Similarly to effects on C uptake, temperature responses of soil CO_2 efflux may differ in the long term. In several studies, soil CO_2 efflux response to warming decreased over time (Atkin *et al.*

2000; Eliasson *et al.* 2005; Heinemeyer *et al.* 2006), either because of acclimation of root metabolism, depletion of labile soil organic matter pools or thermal adaption of soil microbial communities (Luo *et al.* 2001; Bradford *et al.* 2008). Given the increasing frequency of extreme meteorological events, which are often associated with a decoupling of above- and belowground temperatures, the understanding of both short- and long-term temperature responses appears important for predictions on ecosystem responses to warming.

Overall, our study demonstrates air and soil temperatures effects on C cycling processes and their coupling. Generally, soil temperature affected C fluxes more strongly than air temperature did and metabolization of new C assimilates in roots appeared restricted by low soil temperatures. Variation in soil temperature and its effects on root metabolism is therefore likely to play a major role in cold ecosystems responses to warming. Our results further emphasize the importance of disentangling air and soil temperature variation when assessing ecosystems responses to global change, either by applying warming in field trials, or computing models based on future climatic scenarios.

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Figures

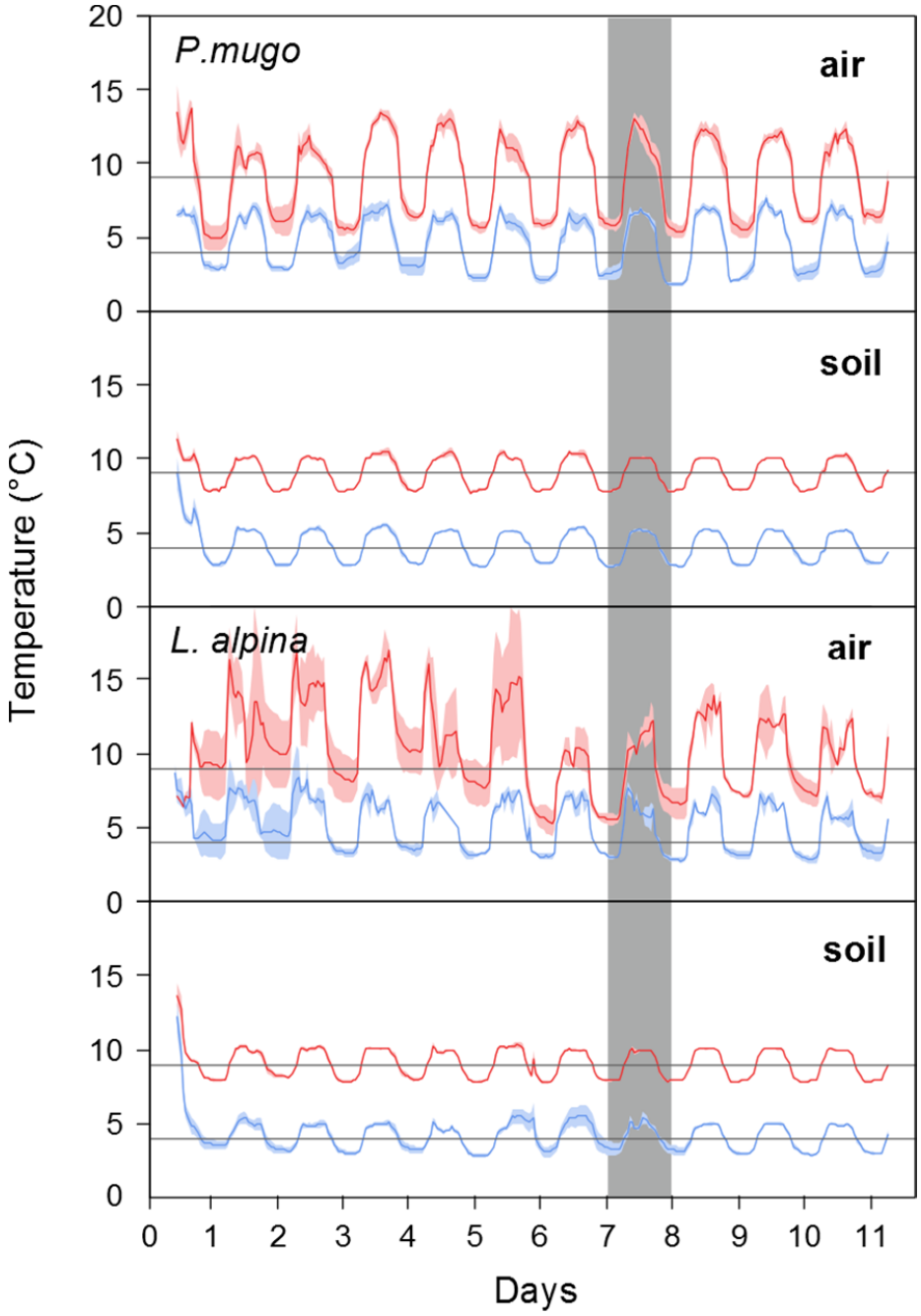

Fig. 1 Air and soil temperature over the 11 days of experimental manipulation. Temperatures are averaged over blocks. Shaded areas indicate standard errors, calculated using blocks as replicates. For air temperature, n=2 in *L. alpina* and n=4 in *P. mugo*. For soil temperature, n=8 in *L. alpina* and n=16 in *P. mugo*. The gray area indicates the day of ^{14}C labelling. Dashed lines indicate target average temperatures.

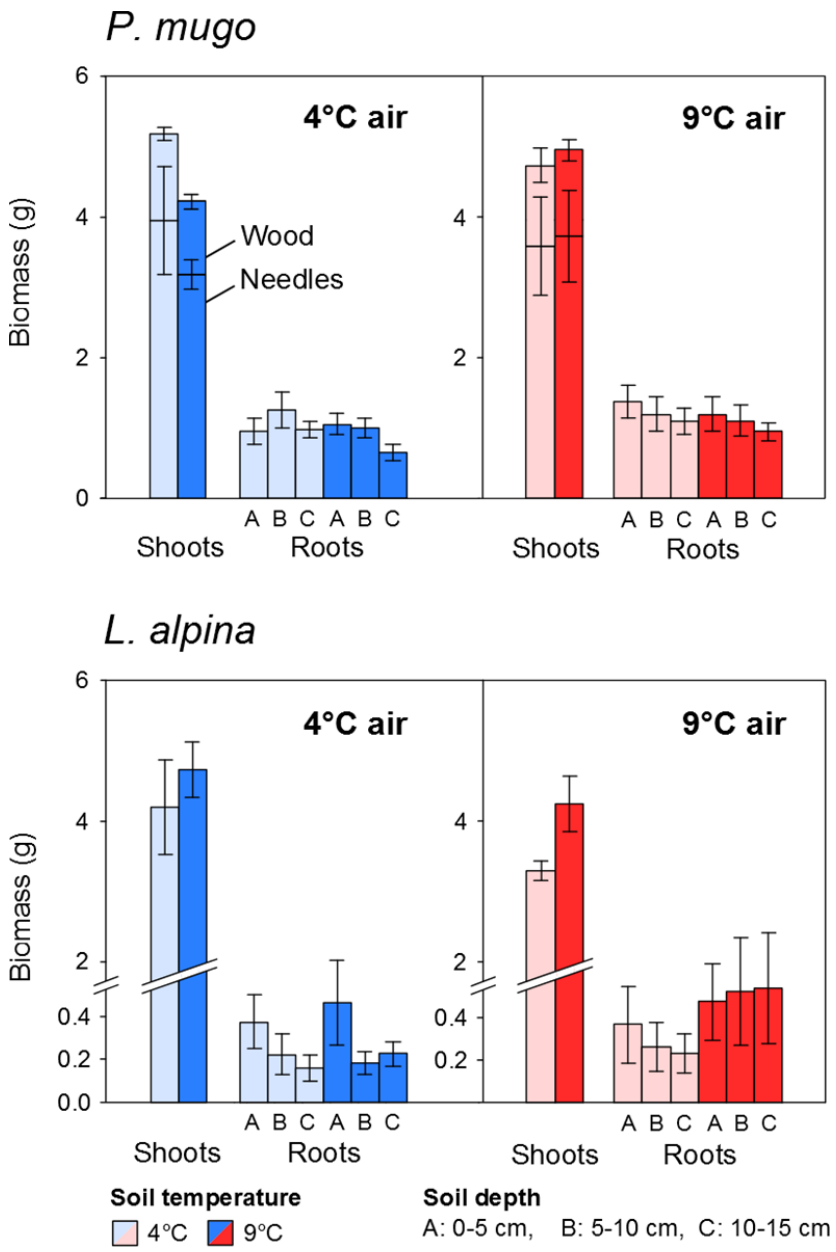

Fig. 2 Combined effects of air and soil temperature manipulation on shoot and root biomass of four-year old *P. mugo* saplings and about 7-month-old *L. alpina* individuals. Error bars are standard errors (n=8 for *L. alpina*, n=12 for *P. mugo*).

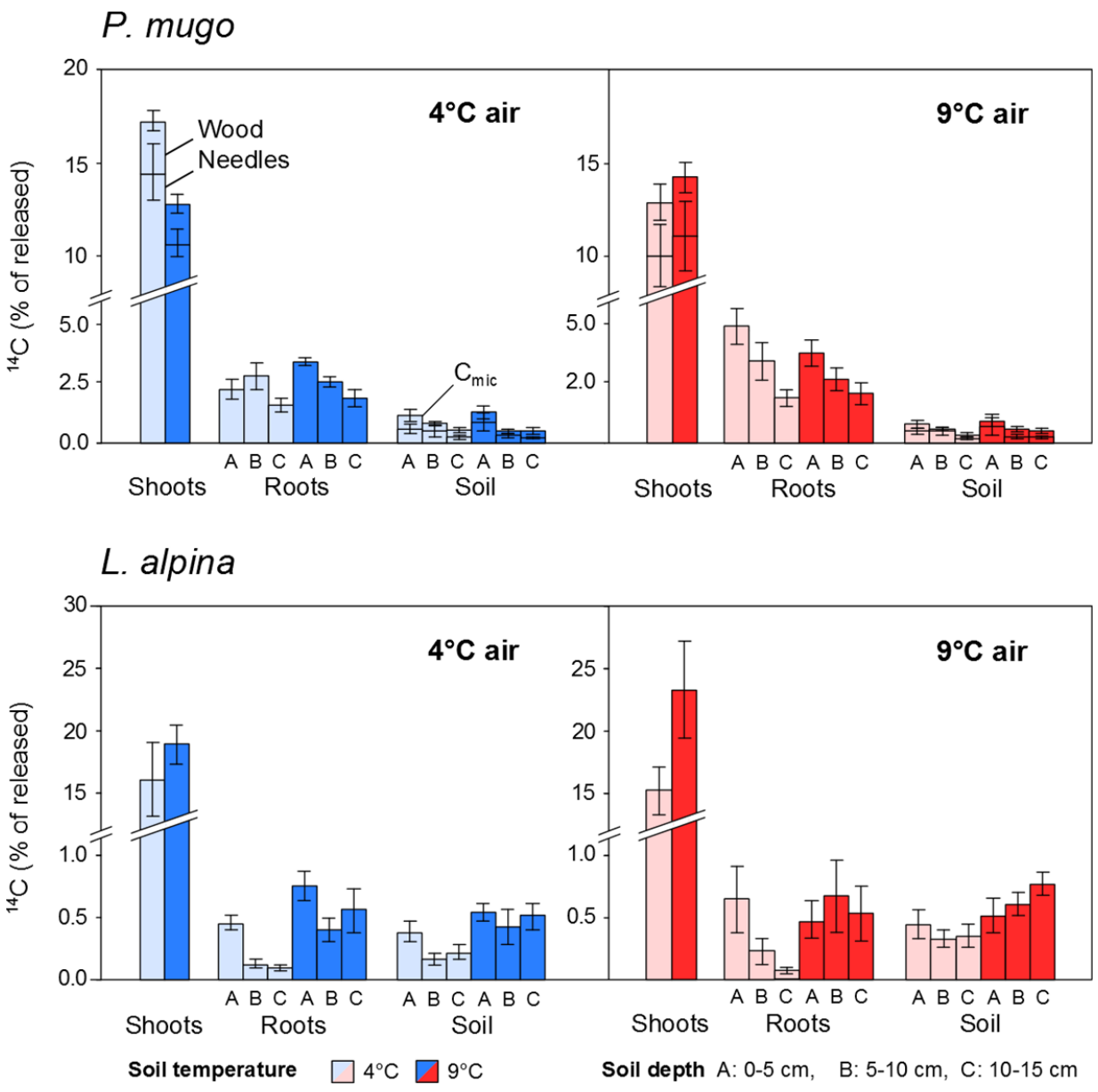

Fig. 3 Combined effects of air and soil temperature on ^{14}C distribution among plant and soil pools in microcosms planted with *P. mugo* and *L. alpina*. ^{14}C activity is shown as percent of released (i.e. if all ^{14}C released was recovered in plant and soil pools, the sum within each panel would equal 100%). Data in each panel are for microcosms labelled simultaneously, thus competing for the same headspace CO_2; e.g. higher ^{14}C assimilation in 9 °C soil would have decreased assimilation in 4 °C soil. For *P. mugo* microcosms, ^{14}C in fungal hyphae was <0.05% and therefore not shown. In *L. alpina* microcosms, ^{14}C in microbial biomass and hyphae were below detection limit. Error bars are standard errors (n=8 for *L. alpina*, n=12 for *P. mugo*).

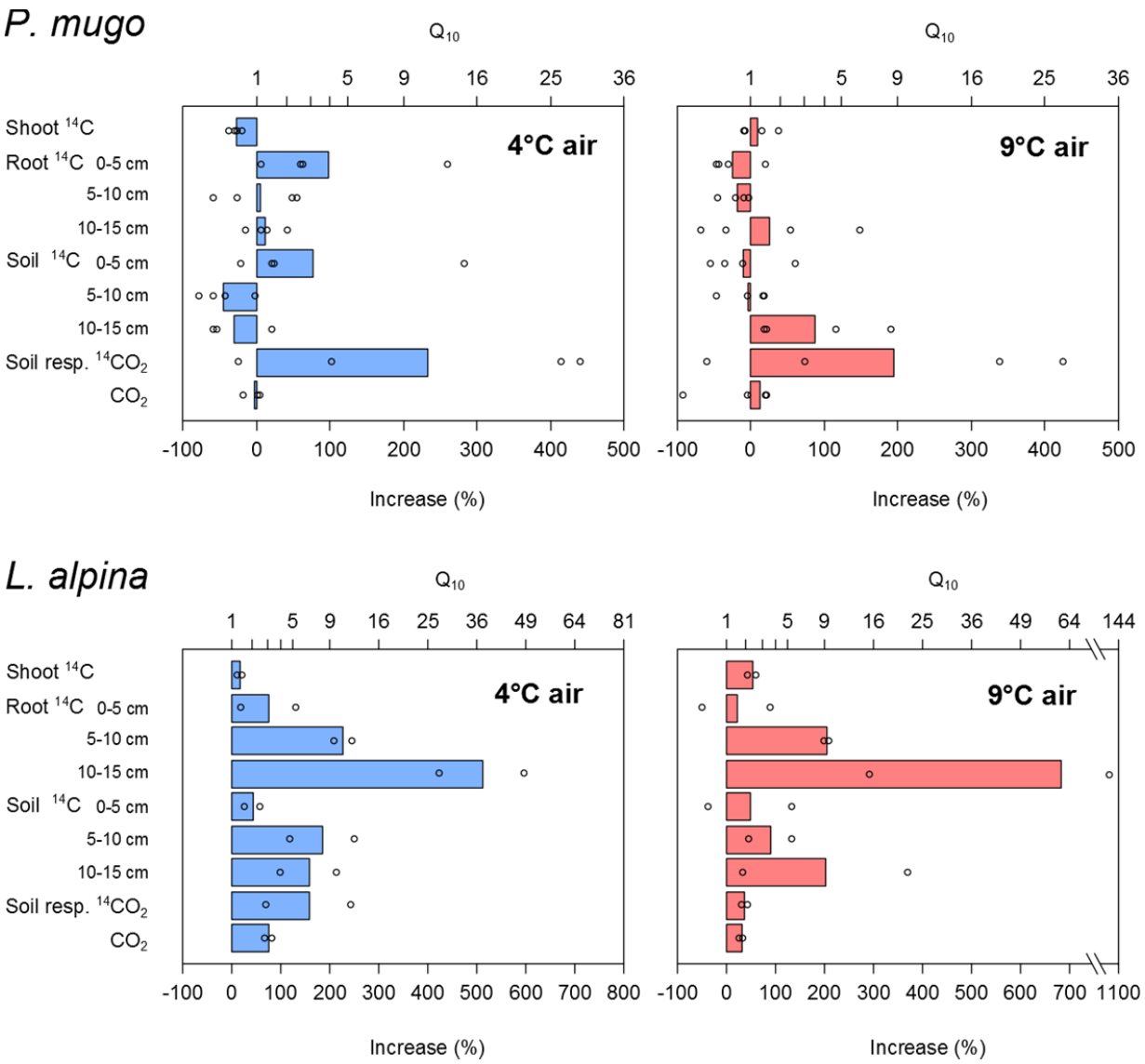

Fig. 4 Soil temperature effect on ^{14}C distribution among plant and soil pools and soil respiration at air temperatures of 4 and 9 °C. Effects are shown as proportional change in ^{14}C activity from 4 to 9 °C (bottom axis) and as Q_{10} (top axis). Root and soil ^{14}C was quantified separately along 3 depth layers. Bars show means while open symbols show average effects per block.

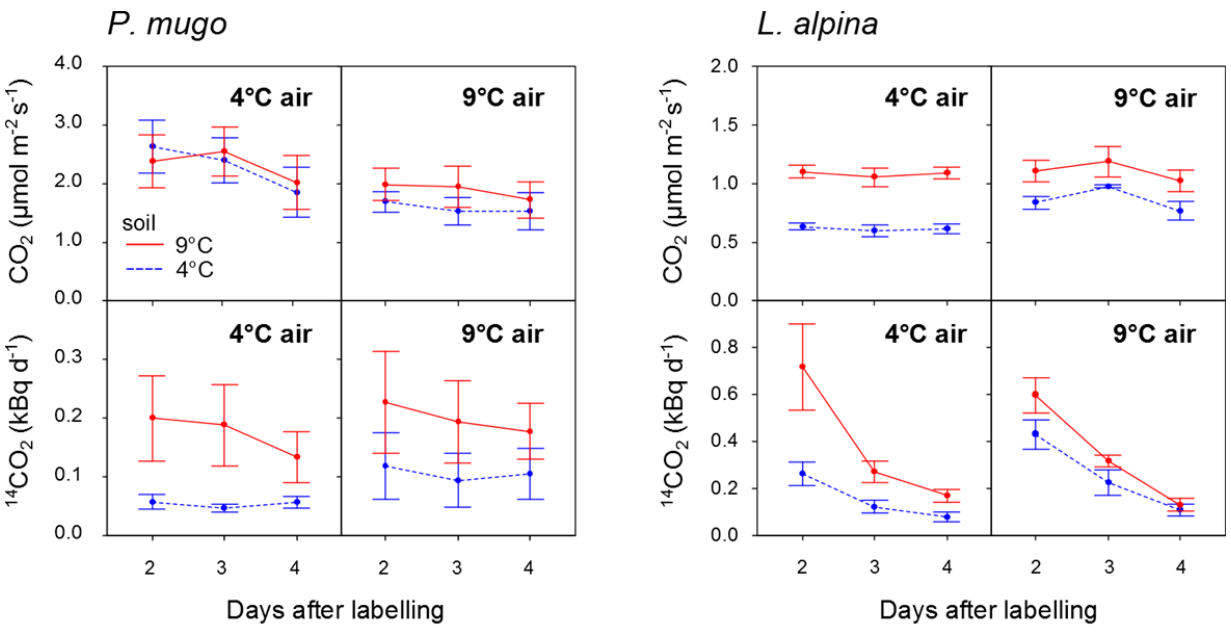

Fig. 5 Air and soil temperature effects on soil CO_2 and $^{14}CO_2$ efflux in microcosms planted with *P. mugo* and *L. alpina* . Soil respiration was trapped over 24h intervals. Data for the first 24 hours after pulse-labelling (day 1) are not available. Error bars are standard errors (n=12).

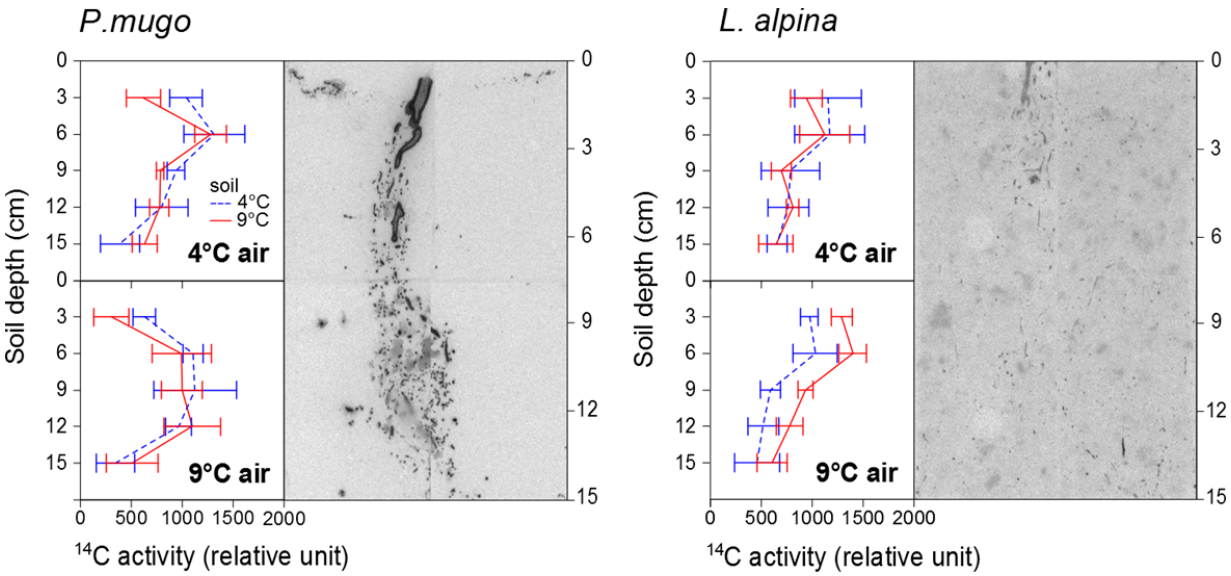

Fig. 6 Autoradiographies showing ^{14}C distribution over the soil profile. Darker pixels indicate higher ^{14}C labelling (right); ^{14}C cumulated in 3 cm layers (left). Error bars are standard errors (n=6 for *L. alpina*, n=8 for *P. mugo*).

Chapter 2

Experimental soil warming and cooling alters the partitioning of recent assimilates: evidence from a ^{14}C-labelling study at the alpine treeline

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Oecologia (in press)

Abstract

Despite concerns about climate change-effects on ecosystems functioning, only little is known on how plant assimilate partitioning changes with temperature. Particularly large temperature-effects might occur in cold ecosystems where critical processes are at their temperature limit. In this study, we tested temperature effects on carbon (C) assimilate partitioning in a field experiment at the alpine treeline. We warmed and cooled soils of microcosms planted with *Pinus mugo* or *Leucanthemopsis alpina,* achieving daily mean soil temperatures (3-10 cm depth) around 5.8, 12.7 and 19.2°C in cooled, control and warmed soils. We pulse-labelled these systems with $^{14}CO_2$ for one photoperiod and traced ^{14}C over the successive four days. Plant net ^{14}C uptake increased steadily with soil temperature. However, ^{14}C amounts in fungal hyphae, soil microbial biomass, soil organic matter, and soil respiration showed a non-linear response to temperature. This non-linear pattern was particularly pronounced in *P. mugo*, with five times higher ^{14}C activities in cooled compared to control soils, but no difference between warmed and control soil. Autoradiographic analysis of the spatial distribution of ^{14}C in soils indicated that temperature effects on the vertical label distribution within soils depended on plant species. Our results show that plant growth, in particular root metabolism, is limited by low soil temperature. As a consequence, positive temperature effects on net C uptake may not be paralleled by similar changes in rhizodeposition. This has important implications for predictions of soil C storage, because rhizodeposits and plant biomass strongly vary in their residence time.

Introduction

Global mean temperatures have increased by almost 1°C since pre-industrial times, most likely as a consequence of anthropogenic activities. Warming is expected to continue in this century, with the largest temperature increases in cold regions, i.e. at high altitudes and at high latitudes (Bradley et al. 2004; Rebetez and Reinhard, 2007; IPCC, 2013). Many physiological processes are limited by low temperatures. Therefore, ecosystem responses to warming, including carbon (C) cycling may be particularly pronounced in cold regions (Rustad *et al.* 2001; Aerts, Cornelissen & Dorrepaal 2006). Despite ongoing research, our capacity to predict climate warming effects on C cycling are limited because an integrated understanding of the mechanisms involved is lacking (Chapin *et al.* 2009; Bahn *et al.* 2010).

Understanding ecosystem-level responses to warming is challenging, partly because individual processes constituting the ecosystem's C cycle are coupled and differ in their temperature sensitivity. For example, plant photosynthesis is strongly controlled by photon flux and thus relatively independent of temperature. Sunlit leaves can therefore maintain positive (although low) rates of photosynthesis even at 0°C air temperature (e.g. Kolari et al. 2007). In contrast, soil respiration responds more strongly to warming, with the soil CO_2 efflux increasing exponentially with soil temperature (e.g. Kirschbaum, 1995). Yet another pattern is found for plant growth. Several studies indicate a discontinuous temperature dependency of root growth and wood formation, which appear to require a minimum (threshold) temperature of 6°C (Körner 1998; Alvarez-Uria and Körner 2007; Rossi et al. 2007).

Another uncertainty is that temperature varies among ecosystem compartments. Especially in forests, canopies very effectively shield soils from solar radiation, therefore decoupling above- and belowground temperatures. Because tree roots appear to require a minimum soil temperature of 6°C to grow (Alvarez-Uria & Körner 2007) and because soil cools with canopy expansion,

closed forest fails to establish in cold environments (Körner 1998). This mechanism has been suggested to determine the altitudinal and latitudinal formation of distinct treelines (Körner & Paulsen 2004).

Given the interdependency of processes and their different temperature sensitivities, warming will not only alter C cycling rates but also C allocation and partitioning among different C pools. A large fraction of soil C inputs occur via rhizodeposition (i.e. root exudation and root litter production), which is tightly linked to belowground C allocation. A critical question is therefore how warming affects soil C inputs and the partitioning of these inputs among soil microbial biomass and soil organic matter fractions differing in characteristics including turnover rates (Chapin *et al.* 2009; Bahn *et al.* 2010; Kuzyakov & Gavrichkova 2010). Across latitudinal gradients, aboveground plant productivity and temperature are correlated (Vogel *et al.* 2008). At the same time, the fraction of C allocated belowground decreases with productivity (Litton, Raich & Ryan 2007) and with mean annual temperatures (Vogel *et al.* 2008; Kane & Vogel 2009). This shift in partitioning among plant compartments might be a response to reduced nutrient availability in cold condition (Giardina *et al.* 2003; Litton *et al.* 2007). Similar dependencies of allocation on temperature have been found under simulated warming with a proportional increase of C allocated to wood and branches and a decrease in allocation to belowground biomass (Savage *et al.* 2013). However, most of these studies were carried out in temperate ecosystems, and little is known about the temperature-dependency of C allocation near the temperature threshold for root growth. For example, many plant species are associated with mycorrhizal fungi, and it remains to be tested whether these symbionts could functionally substitute roots when low temperatures limit root growth (cf. Pumpanen et al. 2012). Such a mechanism could be possible if plants are C sink- (i.e. root growth) rather than source-limited (i.e. photosynthesis) at low temperatures.

Here, we present a study of C dynamics in microcosms subjected to experimental soil warming and cooling in the field near the alpine treeline. Individuals of a woody and an herbaceous species were pulse-labelled with $^{14}CO_2$ and the label traced throughout the plant-soil systems. Specifically, we tested for a soil temperature effect on (1) C partitioning between above- and belowground plant organs, (2) transfer of recently assimilated C to soil organic matter and microbial biomass, and (3) cycling rates of the label through the plant-soil system. Our temperature manipulations covered the soil temperature at the growth limit of trees. We therefore also checked whether (4) the parameters investigated change linearly with soil temperature, or whether non-linear phenomena (e.g. threshold responses) occurred. Although not the primary objective of our study, we further were interested whether responses differ between species.

Materials and Methods

Experimental design

We studied C cycling and partitioning of new assimilates among pools in a field experiment in the Swiss Central Alps at Stillberg (46°46'20" N, 9°51'56" E, 2280 m a.s.l., ≈200 m above the local treeline; see Hagedorn et al. 2010 for details). We manipulated soil temperature in microcosms containing the tree species *Pinus mugo* subsp. *mugo* (Turra) or the forb species *Leucanthemopsis alpina* (L., Heywood). Both species frequently occur at our study site and are quite typical for this type of environment. Microcosms were made from 10 cm diameter×10 cm length polyethylene tube sections that were closed at the bottom with a 2 mm nylon mesh. Microcosms were filled with a mor-type organic layer of a Norway spruce forest (Podzol on non-carbonated sandstone, 1500 m a.s.l.) sieved through a 0.5 cm mesh to remove stones and large roots. The soil was characterised by the following properties: pH ($CaCl_2$) = 2.8, soil organic C =

48.2%, N = 1.7%, C : N ratio = 29.1 (Walthert *et al.* 2003; Pannatier-Graf *et al.* 2011). In each microcosm, we planted either a three-year old *P. mugo* tree, or a three-month old *L. alpina* individual. Both plant species had been grown from seeds collected in the Swiss Alps at altitudes of 2000–2200 m a.s.l.. Pine saplings were about 8-10 cm tall when set in the pots. In addition, each microcosm contained a mesh bag of 3 cm diameter×8 cm length to assess fungal hyphae ingrowth (50 µm nylon mesh, filled with 50 g C-free quartz sand to allow quantitative extraction of hyphae; adapted from Wallander et al. 2001).

At the end of June 2012, shortly after snowmelt, six blocks of 100×50 cm were excavated to a depth of 15 cm and the circumference of each block insulated with 2 cm thick styrofoam boards. Each block was divided into three plots of 33×50 cm each, using the same 2 cm styrofoam insulation. Soil temperature treatments were randomly assigned to plots, with soil temperature being either increased (warming treatment), reduced (cooling treatment), or left unmanipulated (control treatment). The temperature manipulation aimed at cooling or warming soils by about 6°C over the top 10 cm. Warming was achieved with soil heating cables (HD5034, Thermoforce, Cockermouth, Cumbria, UK) spread at the bottom of the plots with 8 cm distance between loops. The electric power deployed in the treatment period was 8.8 W m^{-2}. Cooling was achieved with silicone tubing through which an aqueous anti-freeze solution was circulated using a 20 l cooling bath set at a temperature of -2.5°C (K20-mpc-NR, Huber Kältemaschinenbau GmbH, Germany). Both heating cables and cooling tubing were covered with 2 cm of soil. In the control plots, soil was added to the same level as the treated plots, but remained free of cables and tubing. Six microcosms per plot were arranged on a 2×3 grid, with each plant species being surrounded by neighbours of the other species. The space between the pots was filled with the previously excavated soil material. Finally, the ground was covered with larch litter to protect the bare soil and limit direct warming by solar radiation. To prevent rodent damage, the experiment was

enclosed with an electric fence. Soil temperatures were recorded hourly in one randomly selected pot×treatment×block combination, using automatic data loggers installed at 3, 7, and 10 cm depth (DS1922L, Maxim Integrated, San Jose, CA). Air temperature and precipitation were recorded by the climate station present at the site.

Overall, the experimental design comprised 18 plots for the temperature treatment (6 block×3 soil temperature treatments). Each plot contained three pots per plant species, resulting in a total of 108 pots. Of these three pots, which were pseudoreplicates with respect to the temperature treatment, two were harvested destructively, while the remaining one was used to analyse the ^{14}C distribution using an autoradiographic technique. On June 21, 2012, the soil temperature manipulation was started. Until radiolabelling, soil CO_2 efflux was measured weekly, simultaneously with volumetric soil water content (HH1 soil moisture probe, Delta-T, Burwell, UK) and soil temperature (at 1, 4, 7 and 10 cm depth). Because August 2012 was very warm and dry, we irrigated plots with 4 l m^{-2} on August 13. Climatic conditions during experiment are shown in Fig. 1. Long term annual precipitation and air temperature at the site average around 1100–1200 mm and 2.1°C, respectively (1975–2009, Dawes et al. 2013).

^{14}C pulse labelling

Starting on August 10, the microcosms were labelled block-wise, and soil CO_2 and $^{14}CO_2$ evolution tracked on a daily basis. For the application of the ^{14}C-label, an additional block was set up. This time, however, each plot consisted of a plastic box filled with quartz sand into which the heating cables and cooling tubing were installed. As in the regular field setup, the boxes were insulated laterally with styrofoam. The microcosms were transferred block-wise into the respective plastic boxes, and the entire setup closed with an air-tight acrylic chamber (106×44 cm ground area×56 cm height). A water-filled rim surrounding all plastic boxes served as air-tight

seal. Two heat exchangers fitted with three fans each were installed inside the acrylic chamber, allowing to keep the air temperature below 20°C by manually adjusting the flow from a cooling bath. Treatment position during the labelling was re-randomized for every block by switching the three boxes before every labelling. In summary, this set-up achieved different soil temperatures for each plot, but equal aboveground conditions (temperature, humidity, CO_2 concentration, and ^{14}C activity) since the plots shared a common headspace with forced air circulation.

During the ^{14}C labelling, a pump was circulating air from the chamber's headspace through an infra-red gas analyser (LI-6200, Licor, Lincoln, NE), then through a glass bulb containing $Na_2{}^{14}CO_3$ solution, and then back into the chamber. $^{14}CO_2$ was released by adding diluted sulphuric acid to the $Na_2{}^{14}CO_3$ solution through a septum port. CO_2 concentrations in the chamber headspace were kept between 300 and 500 ppm by releasing CO_2 from unlabelled Na_2CO_3. Microcosms designed for destructive analyses and for autoradiographic imaging were labelled separately, on different days. Per microcosms, 50 or 650 kBq ^{14}C were released for destructive harvest or autoradiographic analysis, respectively. The labelling chamber was kept closed until 18:00 to maximize $^{14}CO_2$ uptake. Then, the microcosms were sealed at the bottom with a thin plastic film and transferred back to their original block. The net amount of $^{14}CO_2$ assimilated during the labelling photoperiod was determined by sampling 300 ml of headspace air with a syringe in the morning, just after releasing the $^{14}CO_2$, and again in the evening, before opening the chamber.

Microcosms designed for plant and soil analyses were harvested five days after start of the labelling (4½ days after the labelling chamber was removed). Half of the microcosms designed for soil autoradiographic analysis were harvested two days after labelling, the other half three days later (i.e. five days after labelling).

Soil respiration and dissolved organic matter

Soil CO_2 and $^{14}CO_2$ efflux were determined using a micro-chamber (a 2.7 cm diameter test tube cut to a length of 6 cm and inserted 3 cm into the soil). Over the 7 weeks preceding the radio-labelling, soil respiration was determined weekly by measuring the CO_2 increase over 60 seconds in a closed system consisting of a membrane pump and a LI-820 CO_2 analyser (LICOR, Lincoln, NE) connected to the micro-chamber. Soil temperature and moisture were recorded simultaneously. During and following the labelling, soil respiration was trapped in a vial containing 2 ml of 1 M NaOH placed inside the closed micro-chamber. The NaOH solution was replaced every 24 hours and analysed later for CO_2 and $^{14}CO_2$. CO_2 was quantified by acid titration of a 0.9 ml aliquot with 0.04 M HCl after precipitation of carbonates with 1.0 ml 0.5 M $BaCl_2$. Trapped $^{14}CO_2$ activity was determined by liquid scintillation counting of a 1 ml aliquot (TriCarb 2900, Packard BioScience, Meriden, CT; 4 ml Ultima Gold cocktail, Perkin Elmer, Waltham, MA).

Dissolved organic carbon (DOC) was collected using 5 cm long samplers (Rhizon CSS, Rhizosphere, Wageningen, Netherlands) inserted vertically into the plant rooting zone (sampled depth range: 2–7 cm). Soil solution was retrieved by attaching a pre-evacuated 12 ml vial which also served as sample container. These vials were replaced every 24 hours and collected solutions kept frozen until analysis. DOC was measured (Dimatoc 2000 TOC analyser, Dimatec Analysentechnik GmbH, Germany) and ^{14}C activity in 1 ml sample quantified by liquid scintillation counting (as described above).

Harvest of microcosms

Microcosms designed for destructive analysis were harvested by cutting plant shoots at soil level. Roots were then separated from the soil and cleaned with water. Hyphal ingrowth bags were

separated from soil and frozen until further analysis. The remaining soil was sieved at 2 mm, and a sample stored at 4°C for microbial biomass determination. Another sample was frozen for total soil ^{14}C analysis. Microcosms designed for autoradiographic imaging were also harvested by cutting shoots at soil level. Then, the remaining microcosm, i.e. the intact soil including the plant root system, was frozen.

Analysis of plant and soil material

Plant shoots and roots were dried at 70°C and weighed (needles and branches of *P. mugo* separately). A subsample of the dried plant material was ground or cut into small pieces (*P. mugo* branches). Subsamples were then combusted (Packard 307 sample oxidiser, Perkin Elmer, Waltham, MA) and the trapped $^{14}CO_2$ activity determined by liquid scintillation counting. Hyphal mesh bags were opened and the content suspended in 250 ml H_2O using a blender. A 50 ml aliquot of the suspension was vacuum-filtered (Whatman 42 filter paper, GE Healthcare, Freiburg, Germany). The filter containing the trapped hyphae was folded, dried at 70°C, combusted in the sample oxidizer and ^{14}C activity quantified as described for plant material. Soil microbial C was determined by chloroform fumigation-extraction (Vance, Brookes & Jenkinson 1987). In brief, a 10 g soil subsample was extracted with 50 ml 0.1 M K_2SO_4 (45 min, table shaker at 150 rpm). A second sample was extracted similarly after fumigation with ethanol-free chloroform. Both extracts were filtered (MN 615 filter paper, Macherey-Nagel AG, Oensingen, Switzerland) and microbial biomass calculated from the total extractable organic C content of fumigated and unfumigated samples (Dimatoc 2000 TOC analyser, Dimatec Analysentechnik GmbH, Germany), assuming an extraction efficiency k_{EC}=0.45 (Wu *et al.* 1990). Soil microbial ^{14}C was determined similarly after analysing the extracts by liquid scintillation counting (1 ml extract in 4 ml UltimaGold cocktail).

^{14}C in total soil organic C, which included fungal hyphae and microbial biomass, was analysed like for plant material, after drying soil at 105°C overnight and sieving through a 1 mm mesh.

Autoradiography of soil sections

The frozen soil columns were freeze-dried and sealed at the bottom with semi-hardened epoxy resin (Laromin C 260, BASF, Ludwigshafen, Germany, mixed at a ratio of 2:3 with Araldite DY 026 SP hardener, Astorit AG, Einsiedeln, Switzerland). After the bottom seal had hardened, the soil core was fully impregnated from the top by adding fresh resin. Trapped air was removed by slowly evacuating the impregnated core in a desiccator to ≈20 kPa and then increasing pressure back to atmospheric levels (Stiehl-Braun *et al.* 2011). The resin was left curing for two days at room temperature and then hardened for 24 hours at 60°C. A 6 mm thick vertical section was cut from the centre of the soil cores using a circular diamond saw. This section was further divided into four quarters which were mounted on glass slides. The surface of these soil samples was ground flat (Discoplan TS diamond cup mill, Struers GmbH, Birmensdorf, Switzerland). Autoradiographic images of the sections were obtained by exposing phosphor imaging plates (BAS III S, Fujifilm, Tokyo, Japan) for five days and scanning at a resolution of 250 µm (Fujix BAS 1000 scanner, Fujifilm, Tokyo, Japan). The images of the four slides were recomposed. Prior to further analysis, the area of the section containing the main root of *P. mugo* was excluded. This step was necessary because the fraction of the highly labelled main root visible in the scan varied depending on where exactly the soil core had been cut. In a first step, the autoradiographic images were assessed visually to detect treatment effects on label distribution. Then, background activities were subtracted and the depth profile of the recorded activity calculated (MATLAB 2012b, MathWorks, Natick, MA).

Statistical analyses

All data were analysed by analysis of variance, fitting linear models reflecting the structure of the experimental design. Data from microcosms containing the same species were averaged per plot prior to analysis (these are pseudoreplicates with respect to the temperature treatment). Plot-level data were analysed using a linear model including block and temperature treatment (aov function of R 3.0, http://www-r-project.org). Hierarchical data (e.g. analysis including both species) were analysed by fitting linear mixed-effects models including plot as random effect (ASReml 3.0, VSNI International, Hempstead, UK). Temperature effects were tested using plot as replicate (n=18), whereas e.g. temperature treatment×species was tested using the plot×species combination as replicate (n=36). In general, data were log-transformed to achieve normal distribution of residuals, and to correct for size differences between plant species (e.g. the null hypothesis underlying tests of species×treatment on the log-scale was that temperature effects are proportionally equal). Shifts in ^{14}C partitioning were tested by analysing the ratios of activities measured in the different components of the plant soil system (e.g. shoots : total plant, or plant : total microcosm). The corresponding models included block and treatment×species as fixed effect, plus plot (block×treatment) as random effect. A significant species×treatment interaction thus indicated a species-specific effect of temperature on the proportional distribution of the label within the microcosm.

In all models, the temperature treatment was fitted as continuous term with three equally-spaced levels, i.e. we tested for linear effects of temperature. This corresponds largely to the achieved soil temperatures (the cooling and warming effects on temperature were approximately equal except for the direction). When data showed a non-linear trend, deviation from linearity was tested by additionally fitting a second-order polynomial. Alternatively, when two temperature treatments showed equal responses and only one treatment differed, a contrast corresponding to

these two groups was fitted. We felt that this approach was more adequate to analyse non-linear temperature effects.

The depth distribution recorded in autoradiographies was analysed by aggregating activities in three depth intervals (0–3.3, 3.4–6.6, 6.7–10.0 cm). Depth-dependent effects were then analysed by fitting mixed-effect models including depth×treatment as fixed effect and depth×plot as random effect.

Results

Soil temperature and moisture

Soil temperature averaged 5.8, 12.7 and 19.2°C in the cooling, control, and warming treatment, respectively (logger data, averaging 3, 7 and 10 cm depth). The treatment effect persisted over time and corresponds to a cooling of 6.8°C and a warming of 6.5°C relative to control plots (Fig. 1). The experimental temperature change decreased towards the surface as the heat energy was added (or withdrawn) at the bottom of the pots (Fig. 2).

Volumetric water contents showed strong temporal dynamics, varying between 12% and 54% throughout the experiment (Fig. 1). Differences in moisture between treatments were comparably negligible (-2.3% water volume in warmed relative to cooled microcosms, $F_{2,10}$=3.40, P=0.07). Soils with *L. alpina* were slightly drier (-2.9% water vol.) than soils with *P. mugo* ($F_{1,15}$=11, P<0.01).

Total C pools

Plant biomass was not affected by the temperature treatments, except for *P. mugo* root biomass which was 29% higher in warmed and control relative to cooled soils ($F_{1,10}$=6.7; P<0.05, Fig. 3).

Microbial biomass decreased with increasing temperature, irrespective of plant species ($F_{1,11}$=16, P<0.01; Fig. 3). DOC concentrations (data not shown) averaged to 51 mg l^{-1}, matching the ranges reported under similar conditions (cfr. Hagedorn et al. 2010), and did not depend on temperature treatments and plant species.

Net ^{14}C uptake

Net amounts of ^{14}C assimilated increased with soil temperature ($F_{1,11}$=51.0, P<0.001), an effect that was more pronounced in microcosms with *P. mugo* than in microcosms with *L. alpina* ($F_{1,16}$=13.2, P<0.01, for species×temperature treatment).

Of the total ≈50 kBq ^{14}C released per microcosm, 5, 13 and 19 kBq were recovered after at the destructive harvest in plant plus soil material of cooled, control and warmed *P. mugo* microcosms, respectively. For *L. alpina*, the corresponding amounts were 13, 18 and 18 kBq. When including ^{14}C in soil respiration, recovered ^{14}C increased to 9, 20 and 27 kBq for *P. mugo*, and 19, 25 and 25 kBq for *L. alpina*. Headspace $^{14}CO_2$ at the end of labelling indicated a net uptake of ≈93% of the $^{14}CO_2$ released. ^{14}C recovery in plants, soil, and soil respiration when the microcosms were destructively harvested five days later indicated that ≈43% of the label was recovered in the microcosms, suggesting that at least 50% of the applied label has been respired by plant shoots between radiolabelling and harvest.

^{14}C labelling of plant and soil fractions

Plant biomass ^{14}C increased linearly with temperature in all fractions except *L. alpina* roots which showed no temperature effect (Table 1, Fig. 4). In soils of *P. mugo* microcosms, ^{14}C amounts in all pools (microbial biomass, hyphae, total soil C) were five times lower in the cooling treatment but did not differ between warming and control treatments (Fig. 4, Table 1). In

soils of *L. alpina* microcosms, no temperature effect on ^{14}C activity was found in any of the pools analysed (Fig. 4, Table 1). ^{14}C activities in collected DOC were below the detection limit of our analytical procedure (<0.5 Bq/ml). We added unlabelled DOC to a known standard to test whether counting efficiency was reduced (quenching), but did not detect such effects. Even if DOC had been labelled to the ^{14}C concentrations found in plant material, the ^{14}C in the analysed solutions would have been at the detection limit of our analysis.

^{14}C partitioning among pools

Partitioning of ^{14}C among shoots and roots in *P. mugo*, quantified as the fraction of total plant ^{14}C recovered in roots, did not depend on temperature. In contrast, the fraction of plant ^{14}C recovered in *L. alpina* roots decreased with temperature (Table 2, $F_{1,11}$=15.2, P<0.01), from 54% in cold soils to 38% in warm soils.

In *P. mugo*, partitioning to soil (expressed relative to the sum of plant and soil ^{14}C) was lower in cooled and warmed than in control soils ($F_{1,10}$=6.9, P<0.05). This non-linear effect resulted from a linear increase of ^{14}C in plants with soil temperature, combined with an increase in soil ^{14}C from cold to control but not from control to warm conditions. No effect on ^{14}C partitioning to soil was found in *L. alpina* microcosms. ^{14}C recovered in fungal hyphae and microbial biomass averaged to 1.6% and 3.4% of total microcosm ^{14}C in *P. mugo* and to 0.3% and 6.4% in *L. alpina* microcosms, with these fractions remaining unaffected by temperature.

Soil respiration

Soil CO_2 efflux increased with soil temperature for both species, both prior ($F_{1,11}$=6.9, P<0.001; data not shown) and following label application ($F_{1,9}$=14, P<0.01; Fig. 5 dashed line).

^{14}C activity in soil respiration increased non-linearly with temperature in both plant species ($F_{1,8}$=10.5, P=0.01). In this light, increases in $^{14}CO_2$ efflux appeared particularly large when moving from cold to control conditions, and increased comparably little from control to warm conditions (Fig. 5, solid line). Relative to the ambient treatment, 84% and 26% less $^{14}CO_2$ were respired in the cooling treatment for *P. mugo* and *L. alpina*, respectively.

Soil $^{14}CO_2$ efflux decreased over the four days following radiolabelling ($F_{1,26}$=109, P<0.001, Fig. 6). The decline of soil $^{14}CO_2$ efflux over the four days following radiolabelling was estimated as first order rate constant in an exponential decay model and did not vary among treatments.

Autoradiographic images

Total ^{14}C activity in autoradiographies (Fig 7) depended on temperature and species (species×temperature: $F_{1,14}$=7.9, P=0.01). Consistent with findings for root and soil ^{14}C activities, total ^{14}C activity increased with soil temperature in *P. mugo* ($F_{1,12}$=14.8, P<0.01), but not in *L. alpina*. ^{14}C distribution along the soil profile depended on the combination of temperature and plant species (species×temperature×depth: $F_{1,64}$=10.8, P<0.002; Fig 7). The shallow *L. alpina* rooting system (reaching 6 to 8 cm depth) resulted in a reduced ^{14}C activity in the lower part of the pot not reached by roots.

When species were analysed separately, ^{14}C in *P. mugo* soils increased with temperature and this effect was larger in the deep soil (depth: $F_{1,32}$=6.9, P=0.01; depth×treatment: $F_{1,32}$=4.6, P<0.05). In contrast, temperature affected ^{14}C distribution over the soil profile in *L. alpina* (depth×treatment: $F_{1,32}$=6.8, P=0.01), but total ^{14}C remained unaffected.

Discussion

Our results demonstrate that, under cold conditions, soil temperature profoundly affects the cycling of new assimilates in the plant-soil system even when air temperature is not manipulated. Net C assimilation strongly increased with soil temperature, and the partitioning of these new assimilates differed between plant species and showed both linear and non-linear responses. The increase in recently fixed C with temperature suggests higher net rates of photosynthesis. However, a direct effect of temperature on photosynthesis (Farquhar, von Caemmerer & Berry 1980; Turnbull, Murthy & Griffin 2002; Grace 2002) can be excluded because air temperature did not vary among treatments. Indirect effects mediated by soil moisture (e.g. via stomatal conductance, Delucia et al. 1991) also appear unlikely since moisture never dropped below 23% of water holding capacity and was only marginally affected by our soil temperature manipulations. Photosynthesis is sensitive to the rate of utilisation and exports of its products (Stitt, Huber & Kerr 1987); therefore, lower C assimilation under cold conditions likely resulted from reduced C sink activities. Such a sink limitation is supported by increased starch concentrations frequently found in plant tissues under cold conditions (Domisch, Finer & Lehto 2001; Kontunen-Soppela *et al.* 2002; Hoch & Körner 2009; Streit *et al.* 2013). This accumulation of photosynthates most likely results from cold-inhibited root growth which abruptly ceases when temperatures drop below a certain threshold, probably around 6°C (Alvarez-Uria & Körner 2007; Hoch & Körner 2009). Although we did not measure starch concentrations, starch accumulation at low temperatures appears to be a fairly general phenomenon. Cold inhibition of root growth or metabolism may therefore explain decreases of net C assimilation with temperature in our study. In line with this presumption, root biomass was lower under soil cooling, at least in one of the species.

Increased ^{14}C uptake at higher temperature could also result from an increased mineral nutrient availability due to accelerated organic matter mineralisation (Melillo *et al.* 2011; Dawes *et al.* 2011). However, nutrients supply to plants was presumably high relative to field conditions even in the cold treatment, because soil sieving likely increased nutrient availability. Plants generally respond to warming by accelerating their development (Arft, Walker & Gurevitch 1999; Domisch *et al.* 2001). Our plants were exposed to manipulated temperatures for approximately seven weeks before labelling and may therefore have been labelled at phenological stages differing in their potential for ^{14}C uptake. Such ontogeny-related effects appear more likely in the deciduous *L. alpina* than in the evergreen *P. mugo*. Accelerated development under warming would have resulted in plants closer to senescence and thus assimilating less C. However, the large positive effect of temperature on assimilation suggests that this either did not occur, or that the effect of ontogeny was comparably small. Conversely, the warming effects on C assimilation we observed may be underestimates.

In line with our findings, many experimental warming studies showed increased aboveground productivity (Rustad *et al.* 2001; Hudson, Henry & Cornwell 2011; Melillo *et al.* 2011; Sistla *et al.* 2013). However, only very few studies report effects on biomass partitioning between shoots and roots, and even less is known about the partitioning of recent C assimilates. In our study, the partitioning of recent C assimilates among roots and shoots varied with temperature in *L. alpina* but not in *P. mugo*. In *L. alpina*, the fraction allocated to roots decreased in the warmed soil; however, this relative effect resulted from increased allocation to shoots rather than decreased allocation to roots. This finding is in agreement with the observations of Paradis et al. (2014) that shoot but not root growth of *Betula glandulosa* seedlings responded to tundra warming using open top chambers. In contrast, Virjamo et al. (2014) found that warming increased root to shoot biomass ratios in *Picea abies* seedlings and argued that plants invested extra resources remaining

after completion of seasonal shoot growth into roots. Similarly, Domisch et al. (2001) reported larger belowground C allocation under soil warming in *Pinus sylvestris* seedlings. Drier soils under warming might explain the increased root : shoot biomass in these studies. Similarly to *L. alpina* in our study, warming increased investment into shoots in other cold-adapted forbs (Hollister & Flaherty 2010).

Our tracing of labelled assimilates are compatible with the idea of a strong reduction of root metabolism and the transfer of recent assimilates to the soil around a critical "threshold" temperature. In *P. mugo*, both accumulation of recent assimilates in soil and respiration of these assimilates did not follow the temperature response of root ^{14}C. The labelling of these fractions increased markedly from the cold to the control treatment, but not further to the warmed treatment, indicating that the additional C assimilated was invested in plant growth, either above or belowground. Soil microbial activity is unlikely to have driven the non-linear responses we observed in labelled respiration. Soil microbes can be active in frozen soils (Drotz *et al.* 2010), and their activity generally increases linearly to exponentially with temperature (Pietikäinen, Pettersson & Bååth 2005). This apparently contrasts the decrease in microbial biomass we found under warming; however, decreased soil microbial biomass under warming has previously been reported and was often accompanied by an increase in microbial activity (Domisch *et al.* 2001; Hagedorn *et al.* 2010; Streit *et al.* 2014). Although temperature-dependent (Gavito *et al.* 2005; Heinemeyer *et al.* 2006; Hawkes *et al.* 2008), mycorrhizal activity has been observed at low (near-freezing) temperatures (Moser 1958). In a soil warming study, Pumpanen et al. (2012) found that – while plant growth and respiration increased with warming – mycorrhizal metrics did not change. We therefore believe that a cold-inhibition of mycorrhizal activity is unlikely, and that root activity and rhizodeposition, rather than microbial metabolism, were limited at low temperatures. Our autoradiographic analyses largely confirmed the results from the harvest data.

In *L. alpina*, no significant temperature effects on belowground allocation (soil plus roots) were found. In contrast, temperature effects increased with soil depth in *P. mugo*, mirroring the magnitude of the applied temperature manipulation. Such differences may result from the two species having different life strategies to cope with cold environments: *L. alpina*, with its shallower rooting system can benefit from warmer topsoil temperatures resulting from direct solar radiation. *P. mugo* roots instead need to grow deeper in the soil to ensure the anchorage of the tree and experience therefore colder temperatures.

Soil CO_2 efflux increased linearly with temperature. The temperature response of soil respiration is the sum of responses of root and the microbial respiration fuelled by both recent assimilates and older C sources. In our study, respiration of recent assimilates ($^{14}CO_2$) responded non-linearly, reflecting the temperature threshold below which root activity and exudation are strongly inhibited. Soil respiration depends on substrate supply (Heinemeyer et al. 2007; Moyano et al. 2007, 2008; Subke et al. 2011), and its response to seasonal temperature variations cannot be separated from indirect effects resulting from other temporal variation in e.g. light intensity and plant phenology (Epron *et al.* 2001; Janssens & Pilegaard 2003). Schindlbacher et al. (2009) therefore concluded that only responses measured under experimental warming reflect the true soil respiration sensitivity to warming. Recent studies applying experimental warming agreed on bulk-soil respiration to be more temperature-sensitive than root and rhizosphere respiration (Hartley *et al.* 2007; Vogel *et al.* 2014; Wang *et al.* 2014). Our findings strongly suggest that the temperature sensitivities of root and rhizosphere respiration depend on the temperature range studied: Warming from low temperatures strongly increased the fraction of recent assimilate-derived CO_2 in soil respiration, whereas warming starting at higher temperatures increased mainly the fraction originating from older C sources. In addition, other factors such as phenological stage (Epron *et al.* 2001) or soil organic matter quality (Leifeld & Fuhrer 2005;

Davidson & Janssens 2006) could be determinant for the response of the two soil respiration components.

Some of the observed effects were species-specific (e.g. recent assimilate partitioning) and generally smaller in *L. alpina*. This may be related to the shallower rooting system of *L. alpina*. The temperature changes induced by our treatment decreased towards the soil surface, so that smaller temperature changes may have been experienced by *L. alpina* roots. This may explain the absence of effects on *L. alpina* root biomass and the absence of threshold-type effects on net soil input of recent assimilates. Nevertheless, effects on belowground C allocation were evident in soil $^{14}CO_2$ efflux data. Warming effects are often species-specific (Hollister & Flaherty 2010; Hudson *et al.* 2011; Dawes *et al.* 2011; Pumpanen *et al.* 2012), but these differences were found to be unrelated to plant functional types in a meta-analysis (Dormann & Woodin 2002). Our results indicate the possibility that such patterns are related to differences in the magnitude of temperature changes, e.g. due to root architecture.

Temperature effects on C allocation depend on the time over which recent assimilates are traced. Our autoradiographies showed that amounts and distribution of label did not change significantly between two and five days. Further, the temporal dynamics of recent assimilates in soil respiration indicated that their turnover rate did not depend on temperature. All of this suggests that the label distribution we found is representative of short-term (days to week) allocation patterns.

In our study, soil temperature treatments were in place for almost two months prior to labelling, i.e. for almost one growing season. In the longer term, warming effects on C dynamics will also depend on factors we did not investigate, including aboveground biomass turnover and changes in vegetation composition (Walker *et al.* 2006; Myers-Smith *et al.* 2011; Sistla *et al.* 2013; Hagedorn *et al.* 2014). Our experiment suggests that rhizodeposition did not increase with

warming, despite increased net ^{14}C assimilation. These disproportionate effects have important implications for soil C storage because turnover rates of rhizodeposits and plant biomass differ (Chapin *et al.* 2009).

In conclusion, our study indicates that warming effects on C cycling strongly depend on the temperature range in which warming occurs. Given the discontinuity of this response, temperature effects on C cycling under cold conditions cannot be predicted based on effects observed under warmer conditions. Our findings further indicate that the critical mechanisms limiting plant growth in cold conditions is effective belowground and likely related to reduced root activity. We therefore speculate that climate change effects on cold ecosystems are driven more strongly by belowground than by aboveground temperatures. This has important implications for the modelling of C cycling under future climatic scenarios, in particular because different changes in air and soil temperature are predicted in a future climate (Jungqvist *et al.* 2014).

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Tables

Table 1 Statistical analyses of soil temperature effects on plant biomass and ^{14}C activity in plant and soil fractions, separate for the two plant species. Effects were tested by linear regression of the response variables against soil temperature (columns named "linear"), and using contrasts comparing cooling treatment with the average effect in warming and control treatments (columns names "threshold"). Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

			P. mugo				*L. alpina*			
Pool	Fraction		linear		threshold		linear		threshold	
			$F_{1,10}$		$F_{1,10}$		$F_{1,10}$		$F_{1,10}$	
C	Plant	Shoot	0.75		1.06		0.10		0.50	
		Root	4.65	.	6.75	*	1.73		0.35	
		Wood	0.36		0.59		-		-	
^{14}C	Plant	Shoot	28.5	***	16.8	**	12.4	**	11.8	**
		Root	48.0	***	35.0	***	0.05		1.04	
		Wood	25.8	***	17.1	**	-		-	
^{14}C	Soil	Microbes	6.29	*	10.2	**	0.001		0.19	
		Fungal hyphae	6.24	*	13.4	**	1.45		0.62	
		SOM	4.63	.	8.24	*	0.01		0.05	

Table 2 Percentage of ^{14}C recovered in plant and soil pools, in dependence of soil temperature treatments.

Plant species	*P. mugo*				*L. alpina*			
Soil Treatment	cold	control	warm	mean	cold	control	warm	mean
Roots	30.7	32.6	35.7	33.0	41.6	40.3	30.3	37.4
Shoots	55.8	40.9	49.3	48.7	35.4	40.5	49.9	41.9
Microbes	2.9	4.5	2.8	3.4	7.2	6.6	5.5	6.4
Fungal hyphae	0.7	2.7	1.4	1.6	0.3	0.4	0.2	0.3
SOM	9.9	19.3	10.9	13.3	15.5	12.2	14.1	13.9

Figures

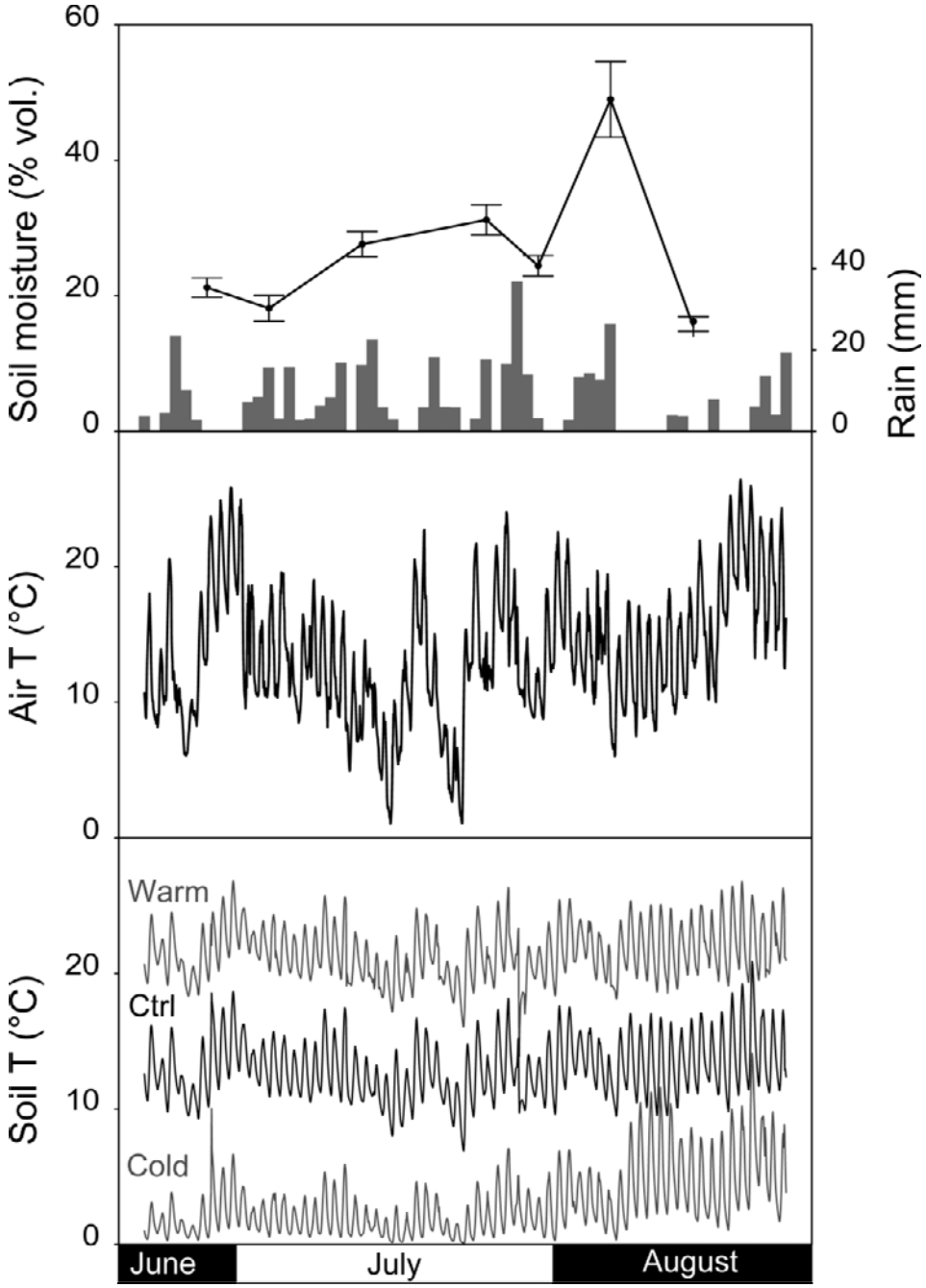

Fig. 1 Volumetric soil water content, precipitation, air and soil temperature over the 57 days of temperature manipulation. Volumetric soil water content was averaged over species and treatments. Error bars show variation among blocks (n=6). Soil water holding capacity is ≈55% vol. Air and soil (10 cm depth) temperatures are averaged hourly and among blocks. Shaded: period of $^{14}CO_2$-pulse-labelling

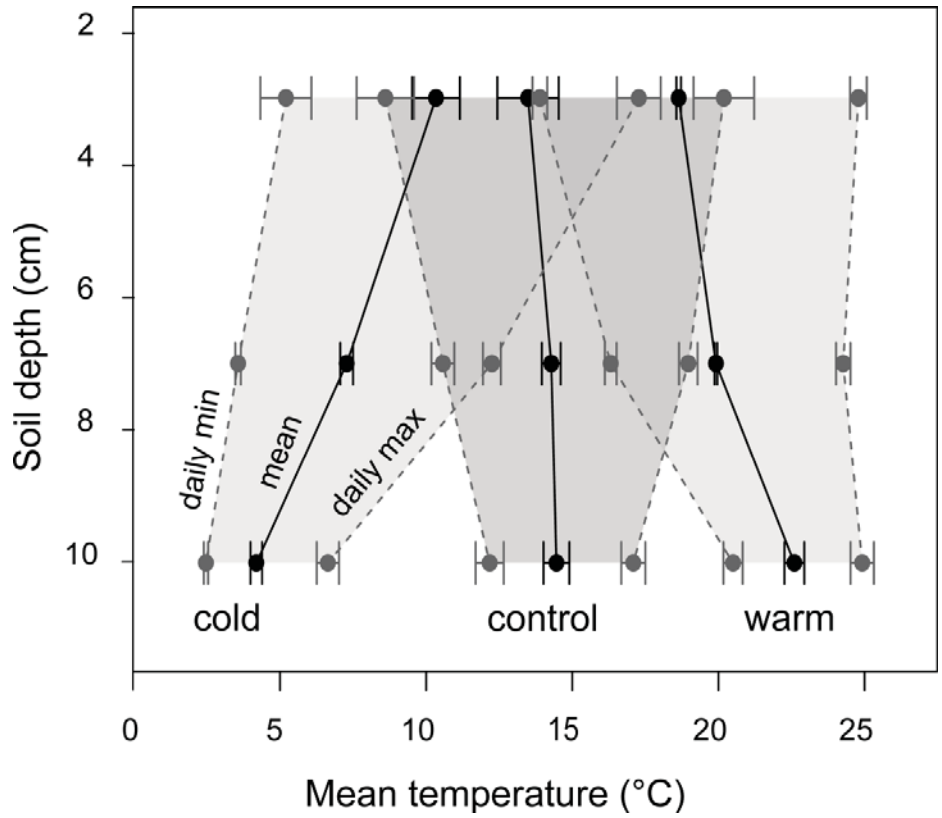

Fig. 2 Daily mean, daily minimum and daily maximum temperature over the soil profile. Error bars are standard errors with block as replicate (n=6)

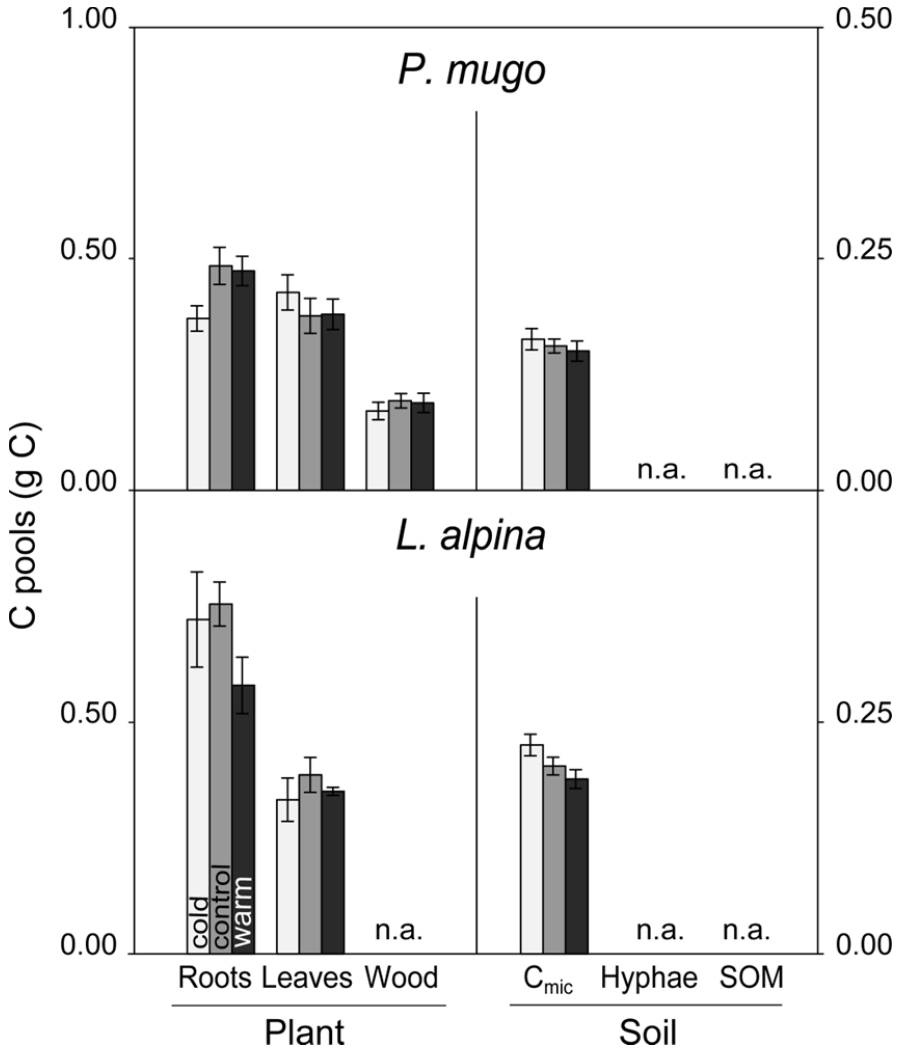

Fig. 3 C pools in microcosms planted with *P. mugo* and *L. alpina.* Soil temperatures averaged 5.9 (cold), 12.7 (control) and 19.2°C (warm). Plant C pools were calculated from biomass assuming a C concentration of 50%. C in fungal hyphae and total soil organic C were not quantified (n.a.). Error bars indicate standard errors across blocks (n=6)

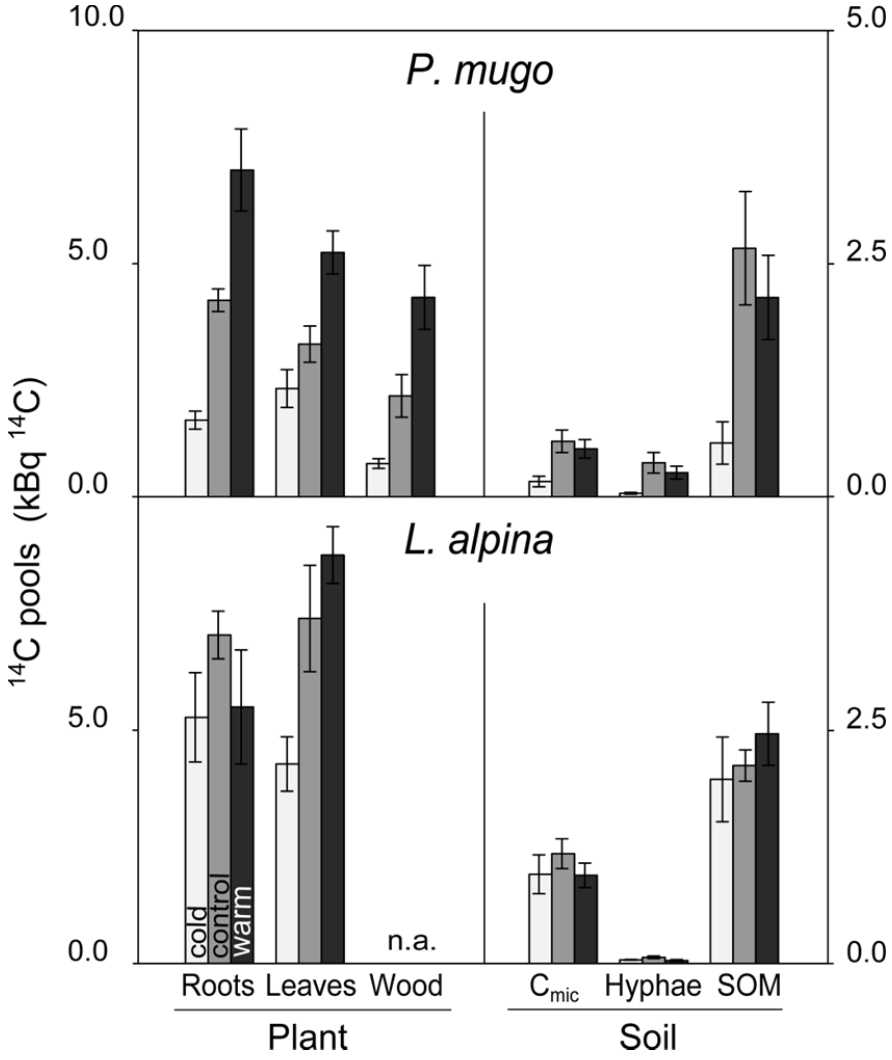

Fig. 4 Soil temperature effects on ^{14}C distribution among plant and soil C pools for microcosms planted with *P. mugo* and *L. alpina* . Note that ^{14}C activities in soil organic matter includes microbial biomass and fungal hyphae. Error bars indicate standard errors across blocks (n=6)

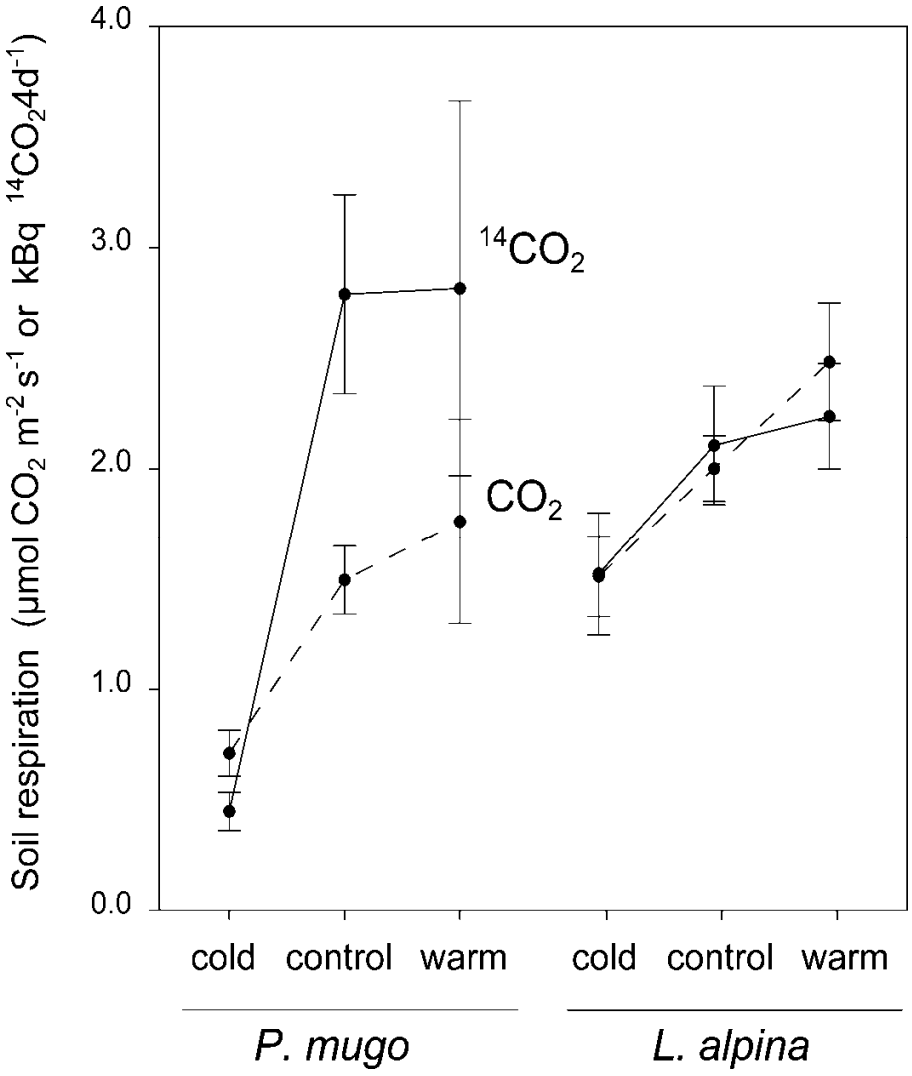

Fig. 5 Soil temperature effects on soil CO_2 and $^{14}CO_2$ efflux. CO_2 efflux is averaged over the day in which radio-labelling occurred and the four following days. $^{14}CO_2$ data are cumulated activities over the four days following the day in which labelling with ^{14}C occurred. All data are on a per microcosm basis, and error bars indicate standard errors across blocks (n=5)

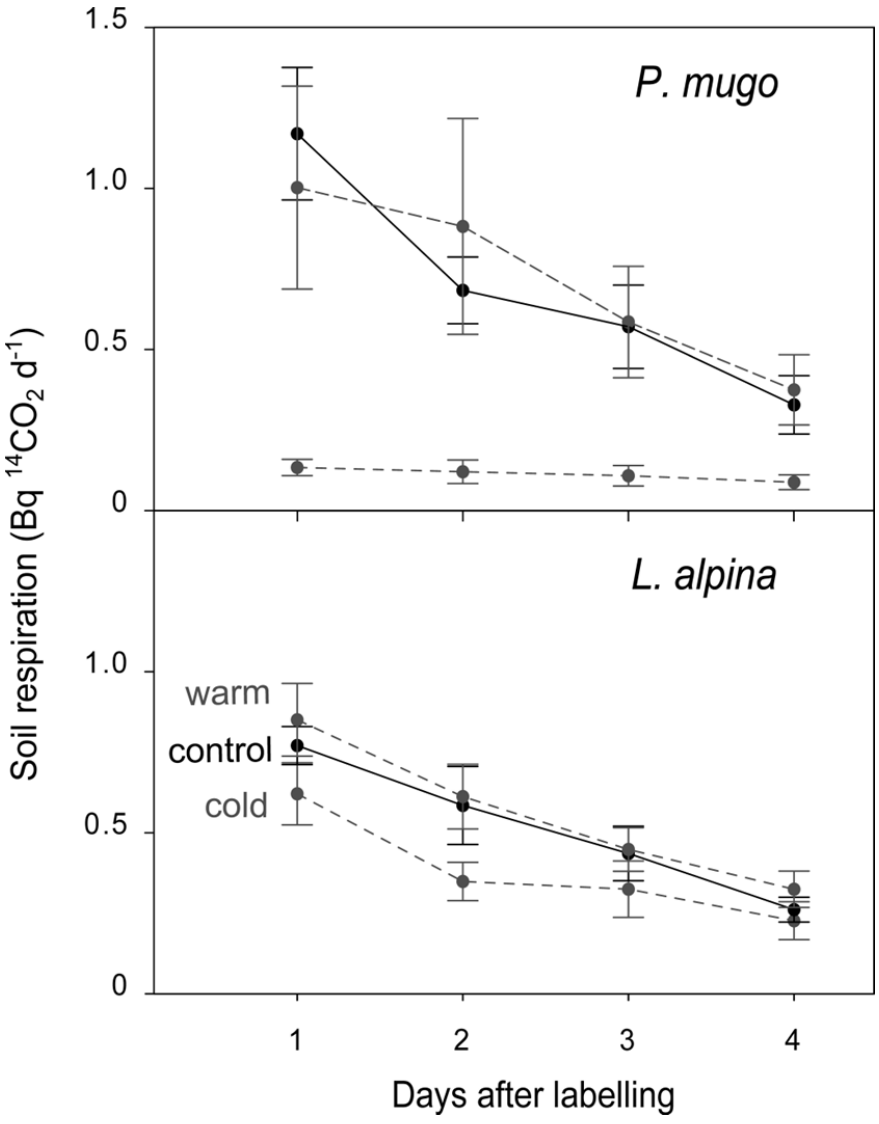

Fig. 6 Soil $^{14}CO_2$ efflux over the 4 days that followed the day of ^{14}C-labelling for *P. mugo* and *L. alpina* exposed to average soil temperatures of 5.9 (cold), 12.7 (control) and 19.2°C (warm). $^{14}CO_2$ efflux for the day of labelling plus the following night is not shown because the NaOH trap likely contained $^{14}CO_2$ label rather than respired $^{14}CO_2$ only. Data are on a per microcosm basis. Error bars indicate standard errors across blocks (n=5, one block removed due to NaOH spill)

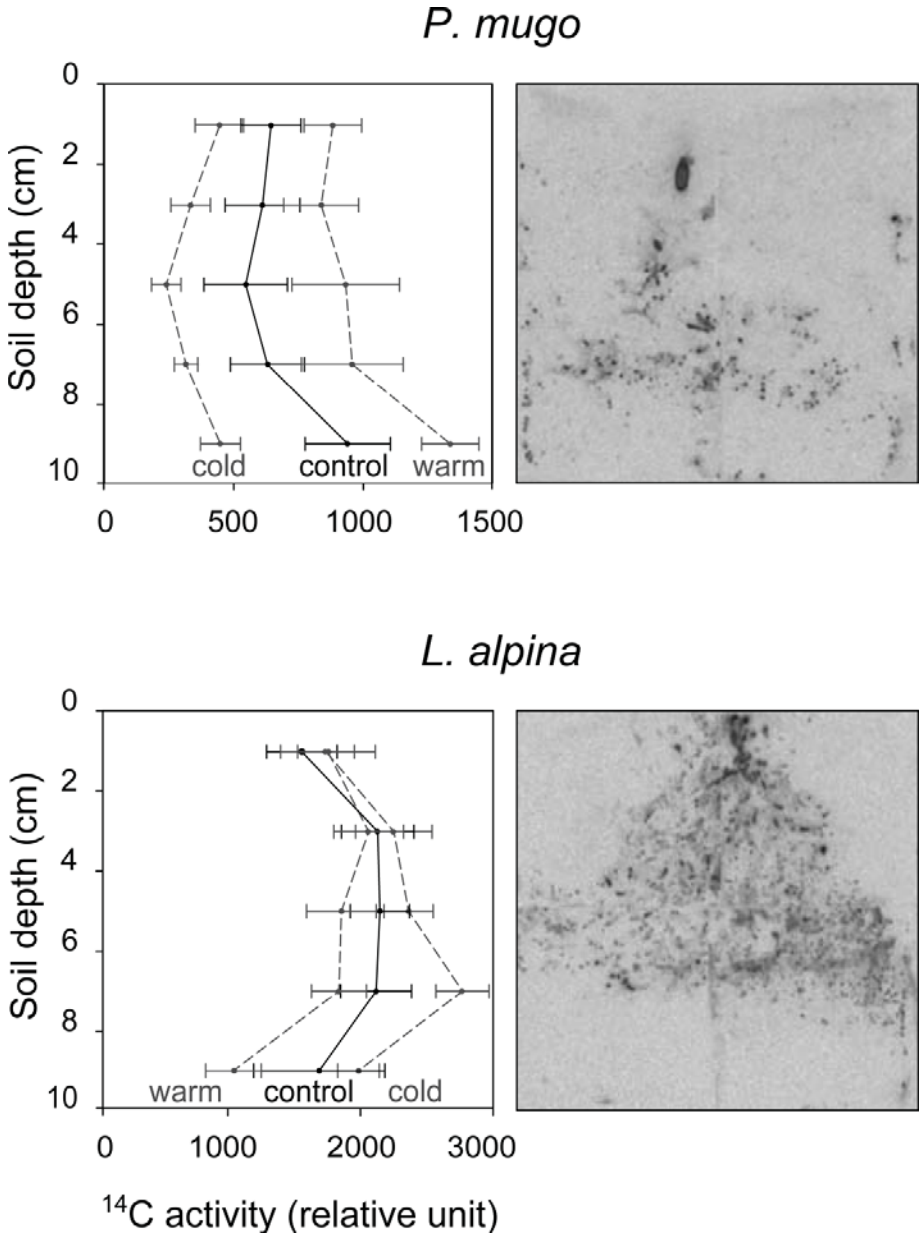

Fig. 7 ^{14}C activity distribution over the 10 cm soil profile in the three soil temperature treatments, at the destructive harvest. Error bars indicate standard errors across blocks (n=6). To the right, an example of an autoradiography for each species is shown, on the same depth scale. Darker pixels indicate higher ^{14}C activity. Note that ^{14}C activity concentrates in roots and rhizosphere without spreading far from the root

Chapter 3

Tracking litter-derived dissolved organic matter along a soil chronosequence using ^{14}C imaging: biodegradation, physico-chemical retention or preferential flow?

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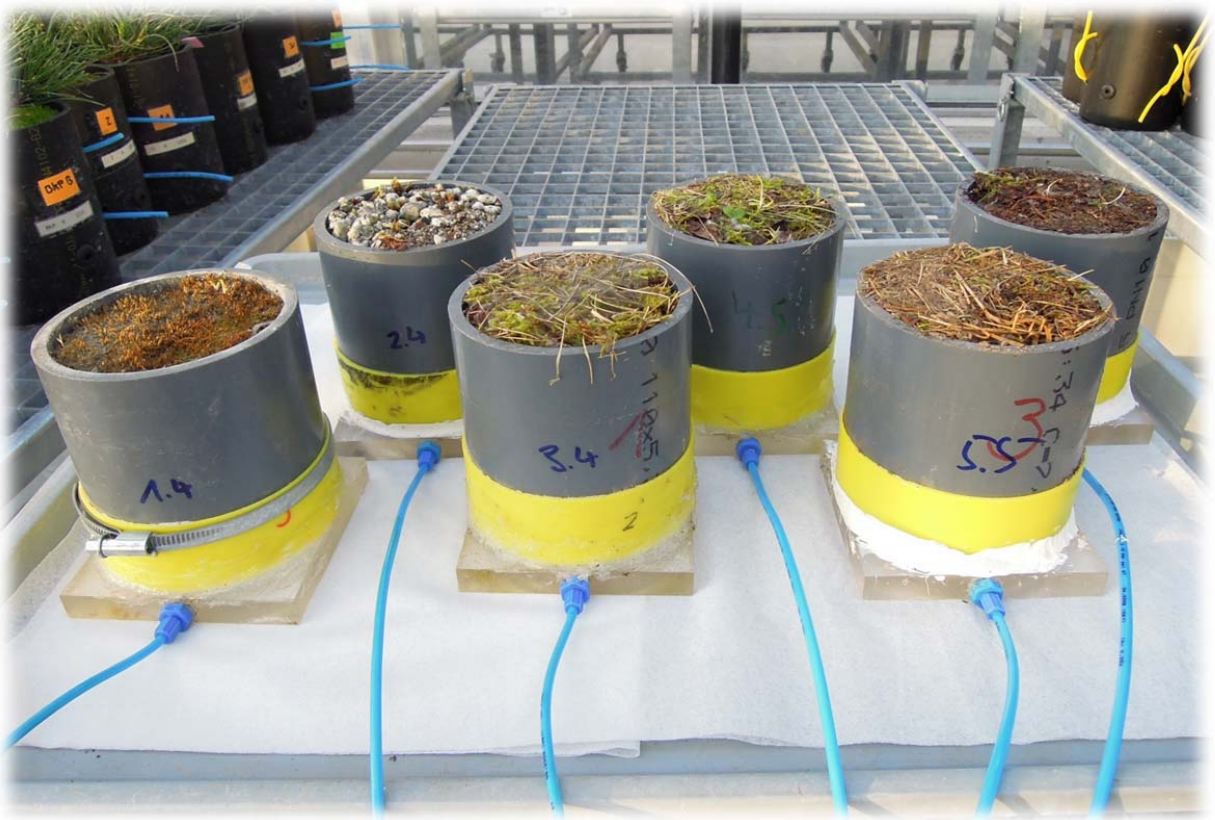

Abstract

The cycling of dissolved organic matter (DOM) in soils is controversial. While DOM is believed to be a C source for soil microorganisms, DOM sorption to the mineral phase is regarded as a key stabilization mechanism of soil organic matter (SOM). In this study, we added ^{14}C-labelled DOM derived from *Leucanthemopsis alpina* to undisturbed soil columns of a chronosequence ranging from initial unweathered soils of a glacier forefield to alpine soils with thick organic layers. We traced the ^{14}C label in mineralized and leached DOM and quantified the spatial distribution of DO^{14}C retained in soils using a new autoradiographic technique. Leaching of DO^{14}C through the 10 cm-long soil columns amounted up to 28% of the added DO^{14}C in the initial soils, but to less than 5% in the developed soils. Biodegradation hardly contributed to the removal of litter-DO^{14}C as only 2 to 9% were mineralized, with the highest rates in mature soils. In line with the mass balance of ^{14}C fluxes, measured ^{14}C activities in soils indicated that the major part of litter DO^{14}C was retained in soils (>80% on average). Autoradiographic images showed an effective retention of almost all DO^{14}C in the upper 3 cm of the soil columns. In the deeper soil, the ^{14}C label was concentrated along soil pores and textural discontinuities with similarly high ^{14}C activities than in the uppermost soil. These findings indicate DOM transport via preferential flow, although this was quantitatively less important than DOM retention in soils. The leaching of DO^{14}C correlated negatively with oxalate-extractable Al, Fe, and Mn. In conjunction with the rapidity of DO^{14}C immobilization, this strongly suggests that sorptive retention DOM was the dominating pathway of litter-derived DOM in topsoils, thereby contributing to SOM stabilization.

Introduction

Dissolved organic matter (DOM) represents the mobile phase of soil organic matter and plays an important role in many biogeochemical processes. Although the fluxes of DOM are small compared to litter inputs and respiration on a local and global scale (Battin et al., 2009; Kindler et al., 2011), the leaching of DOM from topsoils and its sorption in the mineral soil can contribute significantly to the long-term accumulation and preservation of SOM (Kaiser and Guggenberger, 2000; Kaiser and Kalbitz, 2012). In addition, DOM is the major export pathway for essential nutrients such as nitrogen, phosphorous and sulfur from forest ecosystems (e.g. Hagedorn et al., 2000; Michalzik et al., 2001; Kaiser et al., 2003) and it plays a key role in the transport of metals and organic contaminants (Tipping, 2002).

While the fluxes and functional role of DOM have been addressed in a number of studies, the mechanisms behind DOM cycling in soils – mobilization and immobilization – are still not fully understood (Kaiser and Kalbitz, 2012). Principally, DOM derives from leaching of plant litter and microbial detritus, exudation by roots, and solubilisation of soil organic matter, but the relative contribution of each of these sources is still uncertain and differs among ecosystems, soil types and soil depths (e.g. Hagedorn et al., 2004; Sanderman and Amundson, 2008; Malik and Gleixner, 2013). In mineral soils, DOM concentrations typically decrease with soil depth, indicating a net DOM retention. However, different concepts for the underlying retention mechanisms exist among scientific disciplines. A biology-centered view assumes DOM to be highly bio-available, because soil microorganisms take up C in soluble forms (e.g. Kuzyakov, 2010; Kiikkilä et al., 2012) and hence, biodegradation has been assumed to be of primary importance at least for fresh litter leachates (Cleveland et al., 2004; De Troyer et al., 2011). In contrast, organic geochemists consider that DOM is mainly removed in soils by sorption to

reactive surfaces or precipitation, and DOM transport is primarily controlled by physico-chemical properties of DOM (Kaiser et al., 1996; Jardine et al., 1989; Kaiser and Guggenberger, 2000). In a survey of DOM fluxes in 12 European sites differing in land-use, Kindler et al. (2011) observed that the fraction of DOM retained in subsoils correlated positively with the ratio between poorly crystalline Fe and Al-oxides (as a measure of mineral surface reactivity) and soil organic matter (representing a DOM source or the occupation of sorption sites). However, during storm events, the sorptive DOM retention might get by-passed by preferential flow, leading to a more rapid and longer transport than expected based on the surface reactivity of the soil matrix (Hagedorn et al., 2000; Kaiser et al., 2005).

Tracer experiments are powerful to disentangle the retention but also the mobilization mechanisms of DOM in soils. Experimental additions of labelled litter show that only a minor fraction of litter-derived DOM reaches deeper soil horizons, while total DOC concentrations decrease only slightly with soil depth at least in topsoils (Fröberg et al., 2007a; Müller et al., 2009; De Troyer et al., 2011; Kammer and Hagedorn, 2011; Tipping et al., 2012). So far, DOM tracer studies using ^{13}C-labelled organic matter or ^{14}C-'bomb' carbon have been case studies in specific ecosystems and soils (e.g. forests: Fröberg et al., 2007b, Sanderman and Amundson 2008; grasslands: Joos and Hagedorn, 2014). While these experiments all indicate a strong retention of litter-derived 'new' DOM, they have failed to identify the mechanism and spatial dimension of DOM retention as the tracer signal disappears in the large SOM 'reservoir' in soils. The aim of our study was to gain insight into the fate of 'new' litter-derived DOM in soils and to identify the mechanism that control DOM retention. We have set up a soil column experiment, in which we added highly ^{14}C-labeled litter-derived DOM and traced the ^{14}C signal in soil-respired CO_2 and leached DOM, but also in the soil solid phase. In addition, we applied ^{14}C imaging by autoradiography (Stiehl-Braun et al., 2011) to visualize the small-scale spatial distribution of ^{14}C

and hence $DO^{14}C$ retention in soils. For our experiment, we used soils of a chronosequence from a glacier forefield, providing a gradient in soil properties reaching from 'initial' unweathered soils with low SOM contents to 'old' fully developed soils with thick organic layers and high contents of pedogenic oxides (Bernasconi et al., 2011; Smittenberg et al., 2012). We hypothesized that (1) the retention of litter DOM will increase with soil development, (2) biodegradation will increase with soil age as a result of an increasing biomass and diversity of soil microbial communities, (3) the sorptive retention will be highest in intermediately developed soils with the highest contents of potential sorbents, pedogenic oxides relative to soil organic matter, (4) preferential flow leads to a bypassing of the soil matrix, thereby reducing the biotic and abiotic DOM retention.

Materials and Methods

Study sites and soil sampling

We selected soils covering a gradient in soil development from 'initial' unweathered soils to strongly developed soils with organic layers, hence soils that represent a great variability in soil properties potentially affecting DOM cycling. We chose soils from acidic bedrock in the Swiss Alps: five soils of the chronosequence of the Damma glacier (46°38' N, 8°27' E; 1950 to 2050 m a.s.l.) developed from coarse-grained granite (Bernasconi et al., 2011) and one treeline soil (Cambic Podzol), with a thick mor-type organic layer from Stillberg near Davos (46°46' N, 9° 52' E; 2100 m a.s.l.), developed on siliceous Orthogneiss of the crystalline Silvretta nappe (Hagedorn et al., 2008; Müller et al., 2009). The Damma glacier has not receded continuously since the end of the last ice age in 1850 due to glacier advances from 1920 to 1928 and from 1970 to 1992, resulting in two small terminal moraines (Bernasconi et al., 2011). Hence, the soil

chronology consists of four distinct groups of soil ages: <15 y, 60 to 80 y, 110 to 140 y (Hyperskletic Leptosols, WRB, 2006) and a reference site outside the forefield (Haplic Cambisol) with a soil age of at least 700 y. In our study, we have sampled soils from each of these age classes avoiding fresh alluvial material. At the youngest site, we took samples from sandy - frequently flooded- depressions and gravelly mounds (named 10y sand, 10y gravel), which were assumed to have distinctly different flow regimes due to their texture. Moreover, *in situ* measurements have indicated that DOC and CO_2 fluxes differed strongly among these microsites (Guelland et al., 2013a,b). While the vegetation is patchy at the initial sites, grasses dominate at the intermediate sites (named 70y) (*Agrostis gigantea*, *Deschampsia cespitosa* (L.) Roem. and Schult., *Athyrium alpestre* (Hoppe) Milde.). The vegetation cover is full at the older sites (named 120y) with dwarf shrubs such as *Rhododendron ferrugineum* L. and *Salix spp*., and grasses like *A. gigantea*. The reference site of the Damma glacier, the so-called 'grassland' is covered by the grass *A. gigantea* and ferns while the 'treeline' site is dominated by the dwarf shrubs *Vaccinium myrtillus* L., *V. gaultherioides* Bigelow and *Empetrum nigrum* ssp. *hermaphroditum* (Hagerup) Böcher. Details and additional information on climate, vegetation and soil properties can be found in Hagedorn et al. (2008), Bernasconi et al. (2011) and Smittenberg et al. (2011).

In August 2012, we sampled four soil columns at each of the six sites (10y sand; 10y gravel; 70y; 120y, grassland, treeline). The columns, which were located at least 10 m apart, were sampled by hammering PVC tubes of 11 cm height and 10 cm in diameter into the ground and by carefully excavating them with a spade. For transport, they were capped with PVC lids at the bottom and the top and stored at 4°C in a cooling chamber during several weeks until the beginning of the experiments. At all locations, additional soil samples were taken with a steel cylinder of 11 cm height and 10 cm diameter for the analysis of soil properties. The excavated columns were horizontally divided into five 2 cm-soil slices.

For analysis, soils were first dried at 60°C for five days, weighed and then sieved to <2 mm. Stones were weighed and their volume >2 mm was determined by water displacement in a graduated cylinder. Porosity of the soil columns was calculated from bulk density ϱ_B [g cm^{-3}] and particle density ϱ_F [g cm^{-3}] assuming particle densities of 2.65 g cm^{-3} for stones and 1.4 g cm^{-3} for organic matter. Soil pH was determined from the <2 mm soil fraction in 0.01 M $CaCl_2$ with a soil/solution ratio of 1:2. Pedogenic oxides were characterized by extracting them with dithionite-citrate-bicarbonate (Al_d, Fe_d, Mn_d) using the method of Mehra and Jackson (1960) and by NH_4-oxalate (Al_{ox}, Fed_{ox}, Mn_{ox}) according to Schwertmann (1964). In the extracts, metal concentrations were determined by ICP-OES (Optima 3000, Perkin-Elmer, Waltham, MA, USA). For the analysis of soil organic C (SOC) and nitrogen (N) contents, aliquots of the <2 mm fraction were finely powdered with a swing mill (Schwingmühle MM 20, Retsch, Haan, Germany). Samples were then measured with a C-N elemental analyser (CE Instruments NC 2500, ThermoQuest Italia SpA, Radano, Italy).

DO^{14}C leaching experiment

Production of ^{14}C-labelled DOC

^{14}C labeled DOC was extracted from ^{14}C labelled plant material of *Leucanthemopsis alpina,* a typical alpine plant growing at all sites of the glacier forefield and at the alpine treeline. *L. alpina* had first been sown and grown in plastic plant pots (diameter 12 cm) and placed for 2 months in a greenhouse. Shortly before flowering, the plants were labelled with $^{14}CO_2$ in a custom-made gas-tight acrylic chamber (50 cm × 50 cm × 30 cm) capturing 16 pots with four to six plants each. In a first step, air CO_2 concentrations in the chamber were reduced to approximately 200 ppm through photosynthesis. $^{14}CO_2$ was then successively released by adding diluted sulphuric acid to sodium ^{14}C-carbonate (specific activity 40-60 mCi $nmol^{-1}$, 5 mCi, 185 MBq, PerkinElmer,

Waltham, Massachusetts, USA) for an entire photoperiod (~8 h). The total CO_2 concentration of the $^{14}CO_2$-enriched air was monitored with an infrared gas analyser (LiCor 840 Inc., Lymington, USA) and kept under 1000 ppm. Plants were labelled with a total activity of 750 MBq ^{14}C. Plants were left in the chamber for a further two days after label release, keeping CO_2 concentration between 400 and 1000 ppm. A fraction of $^{14}CO_2$ respired was thus re-assimilated by plants, maximizing label incorporation into biomass.

After harvesting the plants, DOC was extracted from 250 g fresh ^{14}C labeled roots and artificial rainwater in a 1:10 weight ratio at 20°C. The extraction was conducted four times with the first DOC extraction lasting 24 h and the following three extractions 48 h. Finally, the extracts were diluted with artificial rainwater to an activity of 1400 kBq l^{-1} and a DOC concentration of 29 mg l^{-1} and to have an elemental composition of precipitation typical for alpine ecosystems (0.5 mg $Na^{+}l^{-1}$, 2 mg K^{+} l^{-1}, 0.46 mg Mg^{2+} l^{-1},0.13 mg Mn^{2+} l^{-1}, 1.44 mg Ca^{2+} l^{-1}, 2.7 mg SO_4^{2-} l^{-1}, and 0.58 mg $Cl^{-}l^{-1}$; electrical conductivity 20 µS; Müller et al., 2009).

DO^{14}C tracer addition

The soil columns in PVC tubes were mounted onto porous borosilicate plates (P4-porous glass plates, diameter 10 cm, 10−16 µm pore size) embedded in acrylic plates. We added a thin layer of quartz sand on the glass plates, tested not to affect DOC sorption to ensure a direct contact of the soil material and the plates. The soil columns were fixed on the porous glass plates by gluing a PVC ring onto the plexiglass using Sika Bond AT Universal and tightening the PVC rings around the columns with clamps. Finally, the porous glass plates were connected to a tube (Festo, PUN-4 x 0.75-BL, 2.6 mm inner diameter) leading to a polyethylene bottle 1 m below the soil core, where the percolate was collected. To ensure a continuous flow of DO^{14}C solution through the

soil columns and to avoid 'air-plugging', we connected the bottles to a vacuum pump (EcoTech, Bonn) applying a constant suction of 10 to 100 hPa.

The litter-derived $DO^{14}C$ solution was added drop-wise to the soil columns with a peristaltic pump (Ismatec IPC-N-24) using three PU-tubes (SMC, TIUB-01-20, 2.0 mm inner diameter) per soil core. These PU-tubes were fixed to PVC lids by silicon plugs at different radii. By rotating the lids in regular time intervals and by adding a 2 cm thick layer of quartz sand on top of each soil column, we avoided splash erosion by water drops and obtained a homogeneous distribution of the added solute, which had been tested with blue color dye prior the main experiment.

The $DO^{14}C$ experiment was conducted in a greenhouse serving as ^{14}C laboratory with air temperatures fluctuating between 15°C and 20°C. The experiment was carried out in two blocks that were processed sequentially. Each block consisted of two of the four replicates per site. The solution was added every 48 hours in cycles by dripping 160 ml of rainwater over 2 hours either with or without $DO^{14}C$. Each application corresponded to 20 mm of rain or 10 mm per day.

The overall experimental addition of solutes was divided into the following four consecutive phases (see Figure 1of supplementary material):

(1) *Initializing*: Addition of artificial rainwater until steady-state was reached where the amount of leached water volume remained constant. This steady-state was reached after three to four leaching cycles.

(2) *Bromide tracing*: In order to investigate the flow regime of each soil column, a bromide tracer was applied as KBr with a concentration of 4 mg Br l^{-1} followed by five additional leaching cycles with 160 ml of rainwater (10 days).

(3) *$DO^{14}C$ application*: Addition of litter-derived $DO^{14}C$ with a total activity of 788 kBq per column during four leaching cycles (8 days). Here, we first added 140 ml of $DO^{14}C$ solution followed by a 'cleaning' with 20 ml artificial rainwater.

(4) *Post ^{14}C addition*: Five leaching cycles during 10 days with 160 ml of artificial rainwater.

The added rainfall rate amounted to 70 mm per week, which is slightly above the average 50 mm of rain per week during the summer months at the Damma glacier with a high total annual precipitation of 2400 mm (Guelland et al., 2013a). During the leaching experiment, soil columns were eluted approximately 12 times in the 10y gravel soils with low pore volumes of 19% and three times in the two old soils with high pore volumes of 68%. We terminated the experiment when ^{14}C activities in respired CO_2 and leached DOC were below 15% of those during the ^{14}C addition and did not change with time.

Measurement of ^{14}C pools and fluxes

DOC leaching. Leachates from the soil columns were collected in two steps. The first sample was taken 100−120 min after the beginning of each leaching cycle when approximately 80 ml had been leached. The second sample was taken after 48 hours. Samples were stored at 4°C. Bromide concentrations were measured from samples of the pre-leaching phase using ion chromatography (ICS-3000, Dionex, Sunnyvale, CA, USA). Total organic carbon (TOC) concentrations were measured with a Shimadzu TOC-V 500 (equipped with ASI-502 autosampler, Kyoto, Japan). The UV absorbance of the leachates as a measure for their aromaticity and contents in the so-called 'hydrophobic' DOC (Chin et al., 1994; Dilling and Kaiser, 2002) was measured at wavelengths of 285 and 260 nm (Cary 50 UV spectrophotometer, Varian, Inc., Palo Alto, CA, USA). Specific activities of $DO^{14}C$ were quantified by liquid scintillation (Tri-Carb 2900TR, Liquid Scintillation Analyzer, Packard BioScience, Meriden, CT, USA) using an Ultima Gold Cocktail (Perkin Elmer, Waltham, MA, USA).

For *C mineralization* measurements, soil columns were closed with custom-built, 5 cm high PVC lids having a rubber seal. Soil-respired CO_2 from each soil column was trapped in 12 ml of 1 M

NaOH in plastic beakers placed in these closed chambers. The trapping solution was replaced after 24 h. Respiration rates were determined by first precipitating carbonates with $BaCl_2$ and titration the excess of NaOH with 0.1 M HCl and phenolphthalein as pH indicator (Alef and Nannipieri, 1995). Specific $^{14}CO_2$-activity was counted by liquid scintillation as described above.

Soils. After the last ^{14}C flux measurement, a soil corer with a diameter of 2 cm was used to collect soil material in one-cm slices from each soil column to quantify the ^{14}C retention in soils. Soil samples were then dried at 80°C during three days and sieved to <1 mm. Approximately 100 mg of sample were combusted in a Sample Oxidizer (Model 307, Perkin Elmer, Waltham, MA, USA). The released $^{14}CO_2$ was analyzed by liquid scintillation counting as described previously.

Autoradiography

Ten days after terminating the $DO^{14}C$ addition, the intact soil columns were frozen at -20°C. freeze-dried during one week and impregnated with epoxy resin (Laromin C260, BASF, Ludwigshafen, Germany, mixed at a volume ratio of 2:3 with Araldit DY 026SP hardener, Astorit AG, Einsiedeln, Switzerland) for autoradiographic images. Prior to resinification, the porous plates underneath the soil columns were replaced by PVC caps to prevent leakage of resin. The infiltration of resin into the soils was facilitated by exposing them to underpressure in a desiccator, followed by bringing them back to atmospheric pressure. Finally, additional resin was added to the columns until it was fully saturated. Resin curing is an exothermic process; to prevent excessive warming during curing, the cores were left harding at 4°C for three days, followed by 20°C for five days, and, in a final step, at 40°C for one day.

The resinified soil columns were cut vertically in half. From one of the halves, a thin slice (<1 cm thickness) was cut off (Discoplan diamond circular saw, Struers GmbH, Birmensdorf,

Switzerland) and subdivided into four similar quadratic pieces. The pieces were mounted onto 3 mm thick glass slides with epoxy resin and polished with a diamond cup mill (Struers GmbH, Birmensdorf, Switzerland) to a planarity of <30 μm. Further details on soil preparation for autoradiography can be found in Stiehl-Braun et al. (2011).

The polished soil sections were placed onto phosphor-imaging plates (BAS III S, Fuji Photo Film Co. Ltd., Tokyo, Japan) which were put in a light-tight box for six days. Imaging plates were then scanned at a resolution of 200 μm (Fujix BAS-1000, Fuji Inc.). To identify specific soil features (roots, stones etc.), all soil sections were also photographed with a digital camera (Nikon Coolpix P300, Nikon, Japan). The four photographic images and the four autographic images obtained from each soil core were digitally recomposed using Adobe Photoshop CS to form a single large image.

Autoradiographic images were first assessed visually to detect variations in $DO^{14}C$ retention along the chronosequence. The vertical distribution of ^{14}C in the soil profile was quantified by first subtracting background intensities of the area surrounding the image using MATLAB 2012b (MathWorks, Natick, MA). Then, we calculated the average and maximum per horizontal pixel line, corresponding to a vertical resolution of 200 μm. Finally, the relative $DO^{14}C$ activity along the depth profiles were estimated by normalizing the values to the mean of all soil column.

Calculations and statistical analyses

^{14}C mass balance. The ^{14}C fluxes and pools were calculated by multiplying measured ^{14}C activities with the corresponding masses (amount of solute and trapping solution as well as soil mass). The mass balance was then calculated either by relating the fluxes to the total amount of added ^{14}C or to the total amount of recovered ^{14}C in leached DOC, mineralized C and soil C.

Statistical analysis of data was carried out by fitting mixed-effect models by maximum likelihood (lme function of the nlme package, R 2.10.1, R Development Core Team (2010)). For the complete data set of ^{14}C fluxes including the treeline site with an unknown site age (probably >5000 years), we fitted *site* and *soil core* as random effect, and *age* as fixed effects. Since the exact age of the reference sites was not known, we assumed 1000 y for the reference grassland site outside of the moraine and analyzed the age effect only for sites of the Damma glacier. For the physical and chemical soil properties as well as ^{14}C activity in different soil layers, we fitted *site* and *soil core* and in addition *site:depth* as random effects using the lme4 package. Similarly, activities obtained from autoradiographic images were analyzed for soil age effects on total $DO^{14}C$ retention and depth distribution over the soil profiles by dividing soils from the glacier forefield into three layers. For the analysis of ^{14}C fluxes, we first integrated the fluxes for each soil column and analyzed the data as described above, but considering that the experiment was carried out in two time blocks. Response variables were log transformed in some cases to meet assumptions of normality and variance homogeneity.

Results

Soil physico-chemical properties

Soil properties reflected the soil development with increasing contents of SOC, decreasing pH values as well as increasing porosity along the chronosequence (P_{age}<0.05; Table 1). For the two initial soils younger than 10 years, the gravel soil on mounds had a much higher stone content (on average: 49 vs. 10 vol %), a higher porosity and higher contents of sesquioxides (P<0.001) than the sandy soil in the depression. Concentrations of oxalate and dithionite extractable Fe, Al and Mn increased with site age in the glacier forefield. However, the site age effect was only

significant for pedogenic oxides when the sandy-depression with alluvial deposits was removed and only the upland sites were included in the analysis (P_{age}<0.05). For the two reference sites, the grassland soil had about two to three times higher contents of oxalate and dithionite extractable Fe and Al than the treeline soil. Soil C stocks of the uppermost 10 cm increased from less than 0.1 kg C m^{-2} in the two initial soils to 1.6 ± 0.3 kg C m^{-2} in the 120 y old soil to 7.1 ± 1.3 kg C m^{-2} in the treeline soil. The two 'mature' soils had similarly high SOC contents in the uppermost two cm of soils (>30% C), but the treeline soil showed a smaller decline with depth and hence, it had about 50% higher SOC stocks in the uppermost 10 cm. Also, soil C/N ratio was higher at the treeline than in the grassland (P<0.001), reflecting the thick mor-type organic layer under ericaceous dwarf shrubs at treeline.

Bromide tracer

The characterization of flow regimes in the soil columns by tracking Br^- showed a rapid Br^- transport in all soils within days except the 10y sandy soil which had a high porosity and a consistent fine textured but layered structure (Figure 1; see Figure 5 for structure). Bromide concentrations in the leachate clearly decreased along the chronosequence (P_{age}<0.01). The peak concentrations were 2 to 3 mg Br^- l^{-1} in the initial soils but only 0.8 mg Br l^{-1} in the two reference soils, which was considerably smaller than the input concentrations of 4 mg Br l^{-1} during one leaching cycle. Accordingly, the Br^- mass recovery decreased with soil age from about 90% at the two 10y old sites to less than 50% in the reference soils (Figure 1), indicating that Br^- was retained in the older soils with low pH values and high contents of Al and Fe oxides. Relating the Br^- breakthrough to pore volumes showed that concentrations peaked 1 to 1.5 pore volumes after Br^- application in the 10y old soils, but already after about 0.5 pore volumes in the older soils (data not shown).

Total DOM leaching and soil C mineralization

As hypothesized total DOM leaching increased with soil age from 2 mg DOC l^{-1} in the 10y old soils to 15 mg DOC l^{-1} in the 120y old soil (before the addition of litter-DOM, Table 2, Figure 2). In the two reference soils, however, total DOM leaching was smaller than in the 120 y old soil of the glacier forefield. Carbon mineralization rates were about one magnitude higher than DOC fluxes. The average mineralization rates increased exponentially from 0.2 to 1.6 g CO_2-C $d^{-1}m^{-2}$ along the glacier chronosequence (P_{age}=0.024). The opposite pattern was found when C mineralization rates were related to the SOC-pool. Then, C mineralization decreased from 3.9 mg CO_2-C kg^{-1} SOC in the 10y soils to 0.15 mg CO_2-C kg^{-1} SOC in the treeline soil (P_{age}<0.01), indicating a decreasing SOM degradability with soil development.

Characteristics of litter-derived DOM and responses of total fluxes

The solution of litter $DO^{14}C$ had a DOC concentration of 29 mg l^{-1}, a DOC/TN ratio of 42, and a specific UV absorptivity at 280 nm (SUVA) of 150 M^{-1} cm^{-1}. The spectroscopic properties if DOM indicates a fraction of the so-called 'hydrophobic' DOM of approximately 30% (calculated based on the measured UV absorbance at 260 nm; Dilling and Kaiser (2002)) and an average molecular weight of 1100 Da (according to the relationship reported by Chin et al., 1994).

Although the solution which was added had a much higher DOC concentration than the leachate from the soil columns, the addition of litter-derived $DO^{14}C$ increased the total DOM leaching only in the two youngest soils (+ 18 mg DOC l^{-1} in the 10y gravel and + 3 mg DOC l^{-1} in the 10y sand), but not in the older soils (data not shown; $P_{site\ x\ litter\ addition}$<0.01). Total mineralization rates remained unaffected by the litter DOM addition in all soils (P>0.2) although the amount of added DOC per leaching cycle corresponded to 0.5 g C m^{-2} and hence, approximately twice the amount of C mineralized per day in the two initial soils.

Leaching and mineralization of litter-derived $DO^{14}C$

The total ^{14}C recovery of the added litter-derived ^{14}C in leached DOM, mineralized C, and soil was 95±8% and did not significantly depend on soil type or soil age. The addition of litter-derived $DO^{14}C$ solution resulted in an instantaneous increase in ^{14}C activities in leached DOM and mineralized CO_2 in all soils, but the ^{14}C activities in C fluxes remained a magnitude below the ^{14}C input of 200 kBq at each of the four leaching cycles (Figure 3). The ^{14}C activity in C fluxes did not increase continuously with repeated ^{14}C addition and showed a characteristic pattern in all soils: respiratory ^{14}C losses were highest at the first day when 'fresh' ^{14}C labelled DOM was added, while $DO^{14}C$ leaching was highest in the second leaching phase following the rapid initial leaching, when the Br^- tracing indicated that 'pre-event' water was displaced by the added solution. After terminating the $DO^{14}C$ addition and adding rainwater onto the columns, ^{14}C activities in C fluxes decreased exponentially to rates smaller than 15% of the ones during $DO^{14}C$ addition (Figure 3).

Integrated over the whole experiment, ^{14}C leaching averaged 9% of the added $DO^{14}C$, whilst ^{14}C losses via mineralization were only 4% (Figure 2). In order to extrapolate the ^{14}C losses to a longer period, we fitted the observed declines in ^{14}C activities after terminating the $DO^{14}C$ addition using exponential models. The extrapolation to one additional month yielded only minimally higher ^{14}C losses with 10% and 7% being potentially lost via leaching and mineralization, respectively.

Leaching and mineralization of ^{14}C showed a significant interaction with soil and soil age, respectively ($P_{fluxtype\ x\ age}<0.001$). While ^{14}C leaching was higher in the young than in the old soils, ^{14}C mineralization increased with site age (Figures 2, 3; Table 2). The leaching of litter-derived $DO^{14}C$ was particularly high in the 10y gravel soil, which also showed the fastest response to the Br^- addition.

Controls of total and litter-derived C fluxes

Total DOM leaching did not correlate significantly with any of the parameters measured (Table 2), while total C mineralization was positively related to SOC contents (r^2=0.72, P<0.007). Leaching of $DO^{14}C$ correlated negatively with increasing contents of oxalate extractable Fe, Al and Mn following a two-pool exponential model (r^2=0.80, P<0.001). However, it was also negatively related to SOC concentrations (r^2=0.56, P<0.005), pH (r^2=0.59, P<0.004) and Br-leaching (r^2=0.68, P<0.001) (Supplemental Materials, Figure 2). The ratio $SOC/(Fe+Al+Mn)_{ox}$ used by Kindler et al. (2011) to predict DOC retention was not significantly related to DOC leaching and $DO^{14}C$ leaching. ^{14}C mineralization was higher in the mature soils, but only tended to be positively correlated to SOC concentrations (P=0.08) and pH values (P=0.07).

^{14}C recovery in soils and autoradiographic imaging

The largest fraction of litter-derived $DO^{14}C$ was recovered in the soil with recovery rates ranging between 70 and 94% of totally recovered ^{14}C (determined by dry combustion). The ^{14}C recovery strongly decreased with soil depth with 50% of total recovered ^{14}C (and 60% of soil-retained ^{14}C) being found in the uppermost 2 cm (Supplementary Materials; Figure 3). While the total ^{14}C recovery in soils did not systematically differ between young and old soils, the vertical distribution differed among soils. The ratio between ^{14}C recovered in the lower and the upper 3 cm of the soil columns decreased from 0.28 in the 10y soils to 0.02 in the 120y soils (P_{age}<0.03), but increased again to 0.08 in the organic layer at the treeline. This indicates that litter $DO^{14}C$ was transported to greater soil depth in the younger soils than in the older soils of the glacier forefield. However, with continuing soil development (and increasing SOM contents), ^{14}C transportation increases again (Supplemental Materials; Figure 3).

In agreement with the dry combustion, autoradiographic images clearly showed a higher ^{14}C activity in the upmost cm of soil, indicating a strong retention of litter-derived $DO^{14}C$ in all soils (Figure 4). Underneath the surface soil, the distribution of ^{14}C activity was highly heterogeneous with high ^{14}C enrichment in individual pores in all soils except the treeline soil. The 10y old sandy soil also showed high ^{14}C activities along lines in-between sand layers and textural discontinuities. Quantitative analyses of ^{14}C activity confirmed that total recovered $DO^{14}C$ did not vary with soil age, but soil age significantly affected the vertical ^{14}C distribution ($P<0.001$) with a relative higher ^{14}C activity in the deeper soil layers in the 10y old soils (Figure 5). Within the soils older than 10y, the average ^{14}C activity decreased from the upper-third of the soil columns to the lower-third by 70%. In comparison to the averages, maximal ^{14}C activities decreased only by 10% from the upper to the lower third of the columns, showing that individual pores in the lower part of the soils had similar ^{14}C activities than in the surface soils (Supplemental Materials, Figure 4).

Discussion

Strong $DO^{14}C$ retention

Our tracking of ^{14}C labelled litter DOM in space and time clearly showed that only a minor fraction of litter-derived DOM is leached from soils while the largest fraction is retained in the uppermost cm of soils. The effectiveness and rapidity of $DO^{14}C$ immobilization indicate that abiotic physico-chemical retention is dominating over biodegradation. Our ^{14}C flux measurement of litter DOM in soil columns is in agreement with field studies with ^{13}C labelled litter on the glacier forefield of the Damma glacier (Guelland et al., 2013), at the treeline (Müller et al., 2009), but also in forests, grass and cropland (Fröberg et al., 2007a; De Troyer et al., 2011; Kammer and

Hagedorn, 2011; Hagedorn and Joos, 2014). In these studies, leached DOM at 5 cm soil depth is comprised only of 1 to 16% of litter-C although total DOC concentrations did hardly change between the litter layer and the upper mineral soil. Similarly, in our laboratory study, litter DOM at 10 cm depth accounted on average only for 10% of the total DOM leaching. This probably represents an upper value as we applied high precipitation rates (20 mm within 2 hours every 2 days) and relatively high concentrations of litter DOM (29 mg DOC l^{-1}) in particular for the initial soils without a litter layer. In addition, the added litter DOM was characterized by a relatively low specific UV absorbance (SUVA) and a 30% fraction of so-called 'hydrophobic' DOM, which are typical for leachates from litter layers directly after litterfall (Kaiser et al., 2001; Kammer and Hagedorn, 2011). These properties strongly suggest the litter DOM to have a low affinity to mineral surfaces (Kaiser et al., 1996) and a low molecular weight (Chin et al., 1994), potentially leading to higher DOM leaching. On the other hand, DOM with a low SUVA indicates a high biodegradability as compared to other litter leachates (Kalbitz et al., 2003; Hagedorn and Machwitz, 2008).

In principle, the retention of litter-derived DOM is driven by its use by the microbial community and by physico-chemically driven retention and transport processes in soils (e.g. Kaiser and Kalbitz, 2012). The relative importance of these mechanisms is, however, highly uncertain and varies with DOM composition but also with key soil properties such as SOM contents and soil mineralogy (Kaiser and Guggenberger, 2000; Cleveland et al., 2004) and hence it will also change with soil development. In our study, we had selected soils of a chronosequence from a glacier forefield, providing a gradient in soil properties reaching from 'initial' unweathered soils with organic matter contents of less than 0.1% SOC to 'old' fully developed soils with a thick organic layer. Accordingly, the chronosequence represented a strong gradient in microbial activity reflected by the strongly increasing C mineralization rates. Similarly, microbial diversity

and carbon limitation of bacterial growth were found to increase along the glacier forefield and to the treeline soil (Esperschütz et al., 2011; Göransson et al., 2011; Hagedorn et al., 2013). However, parallel to the biotic drivers, the contents of pedogenic oxides and hence, reactive mineral surfaces for physico-chemical sorption increased by more than one magnitude with soil development. Moreover, tracking Br^- indicated that the travel times of solutes slowed down along the chronosequence as a result of an increasing porosity, showing that the reaction time of DOM in soil columns increases. In line with the increasing biological and abiotic reactivity as well as the slower solute transport with soil age, our ^{14}C tracer experiment indicated that the leaching of litter-derived DOM decreased with soil age. However, given that soil parameters increased by more than one magnitude along the chronosequence, $DO^{14}C$ leaching varied very little with less than 10% of recovered ^{14}C being leached from 17 out of 24 soils. Only in the initial soils, particularly in the gravel soil with high flow velocities, the fraction of ^{14}C leaching was higher but still below 30% of recovered ^{14}C. We therefore conclude that in 'normal' developed soils, the greatest fraction of litter-derived DOM will be retained in soils.

What was the dominant DOM retention mechanism?

While ^{13}C-tracer studies are all based on the balance between in and outputs and cannot detect the ^{13}C label in large SOM pools (e.g. Müller et al., 2009; De Troyer et al., 2011), our ^{14}C tracking allowed to localize where litter DOM became 'immobilized' within soils. The ^{14}C imaging gave evidence that the major fraction of added litter DOM was recovered within the uppermost cm of soil, implying that DOM immobilization was extremely effective. In addition, our findings show that litter $DO^{14}C$ must have been immobilized very rapidly as the high precipitation rates led to high flow rates through the columns, which was reflected by the rapid breakthrough of Br^-. About 50% of the recovered Br^- were leached from the 10 cm long soil columns within the first two

days. Concomitantly, 50% of recovered ^{14}C were found in the uppermost 2 cm of soil, indicating that $DO^{14}C$ immobilization must have occurred on a time scale of hours or even faster. The rapidity strongly suggests that abiotic processes but not biodegradation are dominating in DOM immobilization. While simple, low-molecular weight compounds can indeed be degraded within hours (van Hees et al., 2005; Hagedorn et al., 2008), DOM of litter leachates and soil solution, decomposes within weeks or months, even when it consists of a large fraction of 'hydrophilic' carbohydrate-rich compounds as the DOM we added (Kalbitz et al., 2003; Hagedorn and Machwitz, 2008). Consequently, it seems unlikely that biodegradation contributes substantially to the removal of litter $DO^{14}C$ during its rapid passage through surface soils. Our measurements of respiratory ^{14}C losses support this conclusion. ^{14}C mineralization was not (negatively) related to $DO^{14}C$ leaching and ^{14}C mineralization rates (on average 5% of recovered ^{14}C) were too small to account for the immobilization of 90% of litter DOM in soils. In our laboratory experiment without plants, we might have underestimated the decomposition of litter-derived DOM compared to natural soils since microbial activity is fueled by rhizodeposits (e.g. Kuzyakov, 2010). For instance, Boddy et al. (2007) have observed twice as high half-life times for low-molecular $DO^{14}C$ in root-free grasslands soils than under field conditions. In addition to respiratory $^{14}CO_2$ losses, incorporation of litter DOM into microbial biomass could have contributed to the $DO^{14}C$ removal. Published substrate use efficiencies show that per unit mineralized C from lignin and maize residues 0.3 to 1.2 units of microbial biomass are produced within the first days, but the values decline to less than 0.1 in a few weeks (Bahri et al., 2008; De Troyer et al., 2011). Translating these substrate efficiencies to our study indicates that biodegradation (mineralization + incorporation into microbial biomass) could account for not more than 15% of the added litter $DO^{14}C$. Hence, immobilization of $DO^{14}C$ by physico-chemical processes must have been dominating at the weekly time scale of our experiment. However, we

assume that over longer time scales (annual or decadal), the ^{14}C balance between mineralization, leaching and immobilization would have been shifted towards C losses as biodegradation is still ongoing upon sorption but at a much smaller rate (Scheel et al., 2008; Tipping et al., 2012).

The correlation of $DO^{14}C$ leaching with soil properties indicated a close negative relationship with pedogenic oxides, which is consistent with the findings in controlled laboratory experiments that DOM co-precipitates or sorbs to Fe- and Al-oxides (e.g. Jardine et al., 1989; Kaiser et al., 1996), either by ligand exchange (Mikutta et al., 2007), hydrogen bonding and /or van der Waals forces (Tipping and Woof, 1991). Incubation experiments showed that biodegradation of DOM decreases by a magnitude upon sorption (Kalbitz et al., 2005; Scheel et al., 2008), which may explain the rapid decline of ^{14}C mineralization after terminating the $DO^{14}C$ addition in our study. Since sorption to mineral surfaces decreases with an increasing occupation of sorption sites (Kaiser and Guggenberger, 2000), we expected that $DO^{14}C$ immobilization would be small in the 'older' SOM-rich topsoil. Indeed, in the treeline soil with a thick organic layer, $DO^{14}C$ was found slightly deeper in the soil than in the soils from the glacier forefield and more $DO^{14}C$ was leached than in the grassland soil and in the 120y soil. However, still, most of $DO^{14}C$ (50% of recovered ^{14}C) was immobilized within the uppermost two cm. The effective retention, also in the organic layer, could be explained by the significant amounts of oxalate and dithionite extractable Fe, Al and Mn, which very likely result from an input of 'new minerals' by erosion along the steep slopes (>30%) and/or by aeolian deposits. Alternatively, DOM could have been immobilized by 'hydrophobic interactions' or condensation reactions as suggested by Fröberg et al. (2007a), but this mechanism is less certain than the interaction with positively charged mineral surfaces (Tipping et al., 2002). The sorptive retention of DOM in the SOM-rich soils is supported by the low recovery of Br-tracer in the two 'old' reference soils (<50%), strongly suggesting that

negatively charged compounds are sorbed to protonated mineral surfaces in the highly acidic soils with pH values below 3.5.

Preferential flow

Despite sorptive retention of DOM, preferential flow could transport DOM into deeper soil horizons (Marin-Spiotta et al., 2012). During stormflow, DOM leached from surface soils bypasses a large fraction of the soil matrix, thereby minimizing the time and surfaces for DOM to interact with oxides and hydroxides (Hagedorn et al., 2000; Kaiser and Guggenberger, 2005). The ^{14}C imaging in our study clearly shows a highly heterogeneous distribution of ^{14}C below the uppermost cm, resulting in similar patterns than in field studies at the profile scale after the application color dyes (e.g. Flury et al., 1994). We relate the spot-wise ^{14}C distribution in all soils to $DO^{14}C$ transport through pores and the ^{14}C distribution along lines in the initial sandy soil to flow along substrate heterogeneities such as differently textured sand layers. All these flow patterns document a bypassing of the largest fraction of soil which potentially accelerates transport velocities and distances. However, $DO^{14}C$ immobilization was strong in all soils, except in the initial gravel soil with 50% stone contents and very low contents of pedogenic oxides.

Implications

Our ^{14}C tracer experiments clearly documents that litter-derived DOM is retained within the uppermost cm of soil. Physico-chemical retention by sorption or precipitation appears to be the dominating immobilization process, reducing DOM bio-availability by ‘sorptive stabilization’ (e.g. Kalbitz et al., 2005; Mikutta et al., 2007). Concomitantly, non-litter derived DOM was continuously leached from soils, which reflects an ongoing generation of DOM during the microbial processing from ‘older’ inherent SOM. These findings contradict the view of

chromatographic stripping of specific components of 'new' DOM leached from forest floors during transport through soils with DOM in deeper soils being the remaining fraction of 'new' DOM after sorption (Kaiser et al., 1996). Our ^{14}C based study rather supports the conceptual model of a 'DOM cycling downward' in soils (Kaiser and Kalbitz, 2012). After a temporal immobilization via sorption or precipitation, retained DOM becomes microbially processed before the transformed compounds are released into soil solution by desorption or dissolution (Malik and Gleixner, 2013).

The ^{14}C imaging revealed a spot-wise ^{14}C distribution along pores underneath the surface soils reflecting preferential (or macropore) flow. The fact that sorbed DO^{14}C is 'immobile' and preferential flow paths may persist for decades (Hagedorn and Bundt, 2002) strongly suggest that the largest part of the soil, the soil matrix, is not actively involved in the processing of 'new' organic matter inputs via leaching. In contrast, macropores will be the 'hot-spots' of SOM cycling, where SOM starts to accumulate and soil microorganisms are permanently fueled by new incoming substrates.

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Tables

Table 1 Soil properties along the chronosequence of the forefield of the Damma glacier and of the treeline site at Stillberg, Switzerland.

		Stone content $cm^3\ cm^{-3}$	Porosity $cm^3\ cm^{-3}$	pH	SOC[a] %	C/N mass ratio	Al_D[b] $g\ kg^{-1}$	Fe_D[b] $g\ kg^{-1}$	Al_{Ox}[c] $g\ kg^{-1}$	Fe_{Ox}[c] $g\ kg^{-1}$
Damma	0–2 cm	0.00	0.47	4.58	<0.1	n.d.	1.07	5.84	0.17	0.56
10 y sand	4–6 cm	0.01	0.30	4.76	<0.1	n.d.	1.25	7.50	0.21	0.69
	8–10 cm	0.16	0.21	4.91	<0.1	n.d.	0.65	3.48	0.16	0.42
Damma	0–2 cm	0.58	0.25	4.46	<0.1	n.d.	0.42	2.32	0.07	0.16
10 y gravel	4–6 cm	0.43	0.30	5.16	<0.1	n.d.	0.22	1.43	0.06	0.13
	8–10 cm	0.48	0.34	5.40	<0.1	n.d.	0.34	1.21	0.05	0.09
Damma	0–2 cm	0.13	0.61	3.95	5.96	15.1	0.98	3.47	0.22	0.39
70 y	4–6 cm	0.17	0.43	4.08	0.36	9.49	0.27	1.97	0.15	0.27
	8–10 cm	0.20	0.41	4.36	0.36	7.87	0.06	1.62	0.09	0.19
Damma	0–2 cm	0.07	0.73	4.14	12.6	18.5	2.38	6.99	0.34	0.64
120 y	4–6 cm	0.13	0.50	3.66	0.84	13.2	1.64	4.83	0.31	0.57
	8–10 cm	0.42	0.36	3.90	0.33	11.2	0.57	2.89	0.19	0.38
Damma	0–2 cm	0.00	0.88	3.82	33.9	18.4	24.9	17.8	3.12	1.49
grassland	4–6 cm	0.01	0.75	3.47	11.7	13.6	17.8	35.7	3.06	2.86
>1000 y	8–10 cm	0.05	0.49	3.47	10.5	12.7	17.5	37.4	2.38	4.45
Stillberg	0–2 cm	0.01	0.88	3.35	34.4	24.6	8.75	14.8	0.93	0.77
Treeline	4–6 cm	0.03	0.70	3.07	22.9	22.7	8.15	17.2	1.07	0.87
	8–10 cm	0.02	0.59	3.07	20.4	23.2	14.1	18.1	1.32	0.84
Significance	Site	0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P-values	Soil age[d]	0.23	<0.05	<0.01	<0.001	n.d.	0.13	0.15	0.072	0.099

n.d. not determined.
[a] Soil organic carbon.
[b] Dithionite-extractable.
[c] Oxalate-extractable.
[d] Tested as log (Age) in the forefield of the Damma glacier.

Table 2 Statistical significances of the linear mixed model testing the effects of site (including treeline), soil age (only glacier forefield) and soil parameters on C fluxes. Linear correlations were estimated when the mixed model indicated a significant effect.

	Total DOC leaching			Total C mineralization[a]			$DO^{14}C$ leaching[a]			^{14}C mineralization[a]		
	F-Value	*P*-value	r^2	F-Value	*P*-value	r^2	F-Value	*P*-value	r^2	F-Value	*P*-value	r^2
Site	45.5	<0.001		99.5	<0.001		18.5	<0.001		4.22	<0.05	n.d.
Soil Age[a]	0.42	0.56	n.d.	18.1	<0.05	0.86	19.9	<0.05	0.53	3.69	0.15	n.d.
Soil organic C[a]	0.12	0.73	n.d.	10.3	<0.01	0.72	11.1	<0.01	0.56	3.49	0.08	n.d.
$(Al + Fe + Mn)_{ox}$[a]	1.57	0.23	n.d.	3.0	<0.05	0.45	44.5	<0.001	0.62	3.30	0.09	n.d.
pH	0.01	0.92	n.d.	60.8	<0.001	n.d.	11.8	<0.01	0.59	3.84	0.07	n.d.
Br	0.01	0.91	n.d.	1.28	0.28	n.d.	81.9	<0.001	0.68	1.05	0.32	n.d.

n.d. not determined.
[a] Tested as log.

Figures

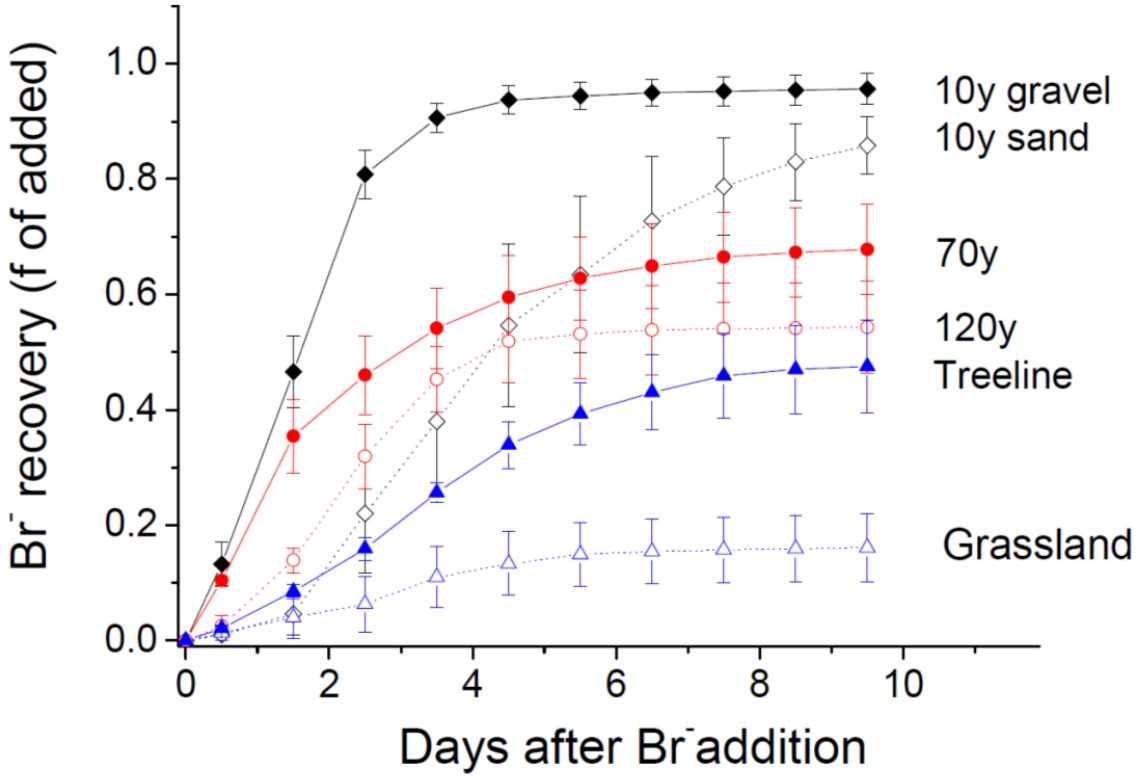

Fig. 1 Breakthrough of Br^- in soil columns of the soil chronosequence to test the flow regimes. Bromide was added as KBr dissolved in 160 ml of rainwater (corresponding to 20 mm precipitation) with a concentration of 4 mg Br^- l^{-1}. After the Br^- addition, rainwater was applied every two days. Means and standard errors of four replicates per soil along the chronosequence.

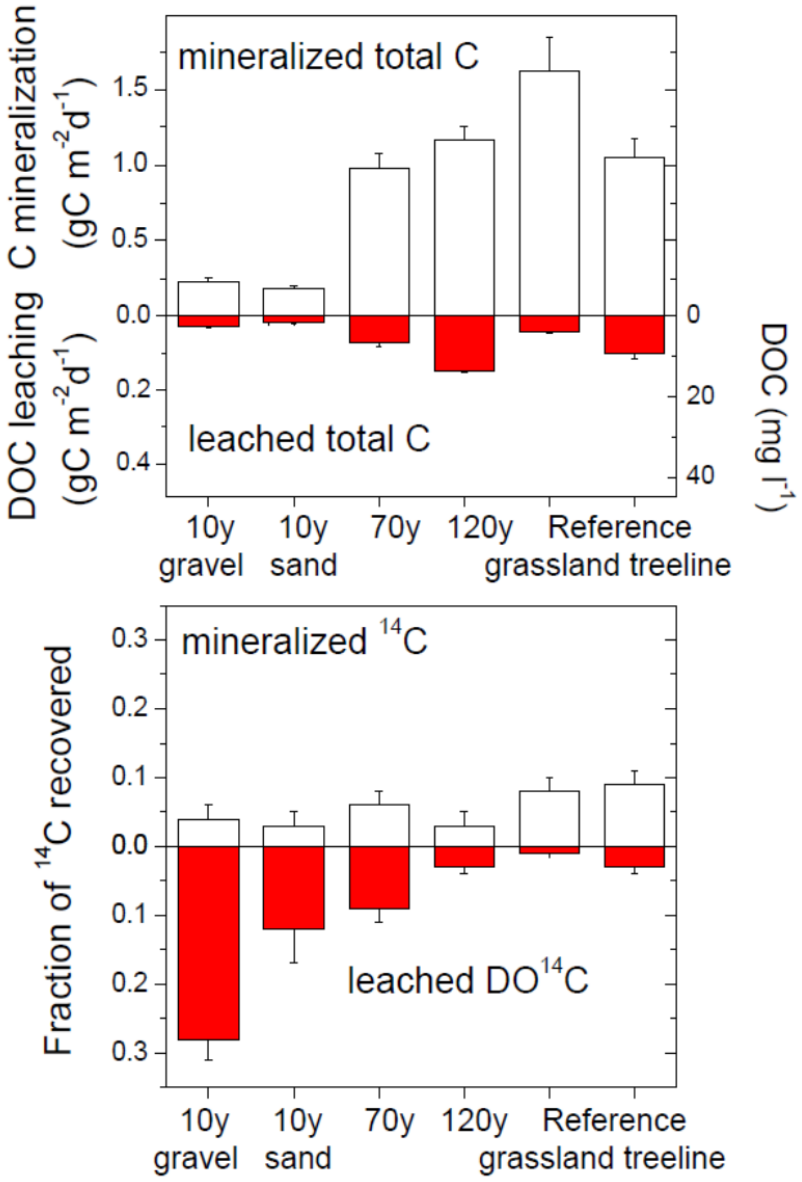

Fig. 2 Mineralization and leaching of total and litter-derived $DO^{14}C$ in the soils along the chronosequence. Note that the total fluxes represent mean daily rates, while the ^{14}C fluxes are cumulated values over the entire experimental period and are presented relative to the amount of ^{14}C recovered. Means and standard errors of four replicates per soil.

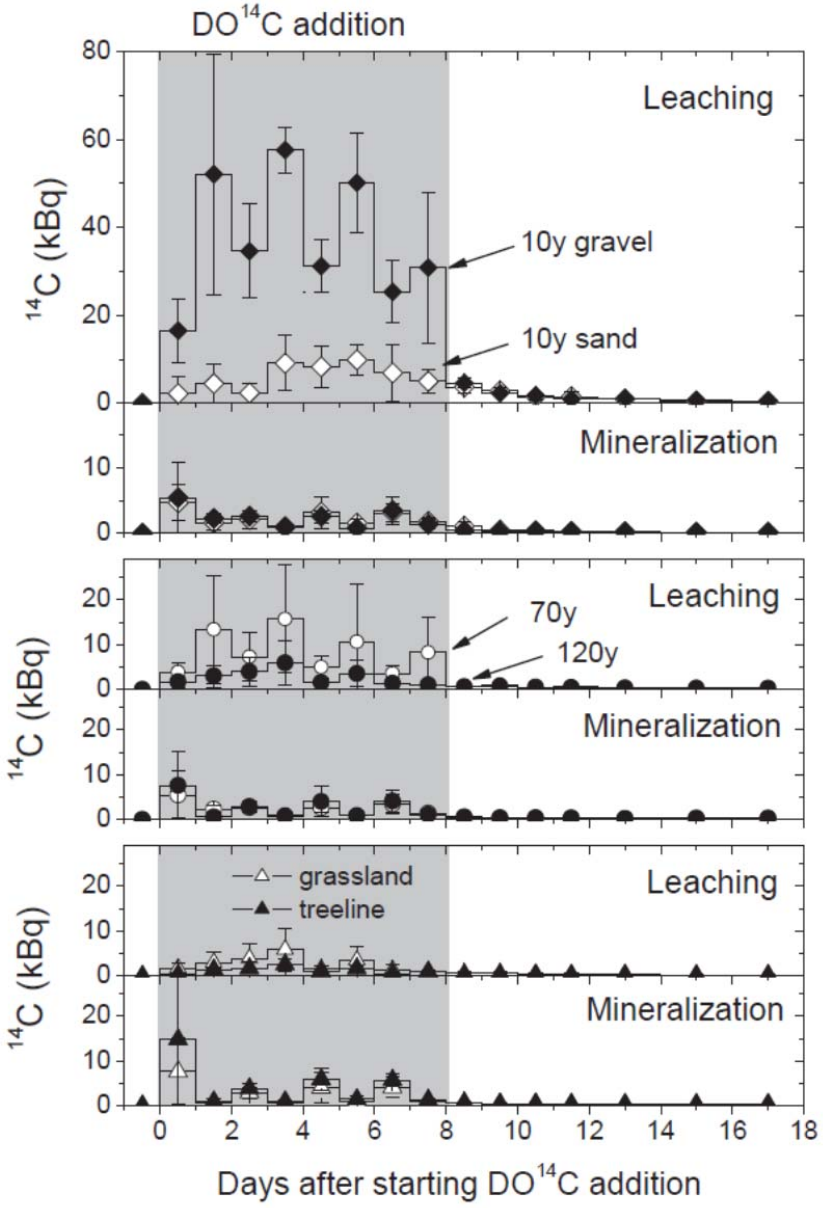

Fig. 3 Mineralization and leaching of litter-derived $DO^{14}C$ in the soils along the chronosequence during the entire experiment. The litter-derived $DO^{14}C$ was added in 160 ml of rainwater (corresponding to 20 mm precipitation) during four leaching cycles (8 days) with the total activity amounting to 788 kBq per column. Thereafter rainwater was added every two days. Means and standard errors of four replicates per soil.

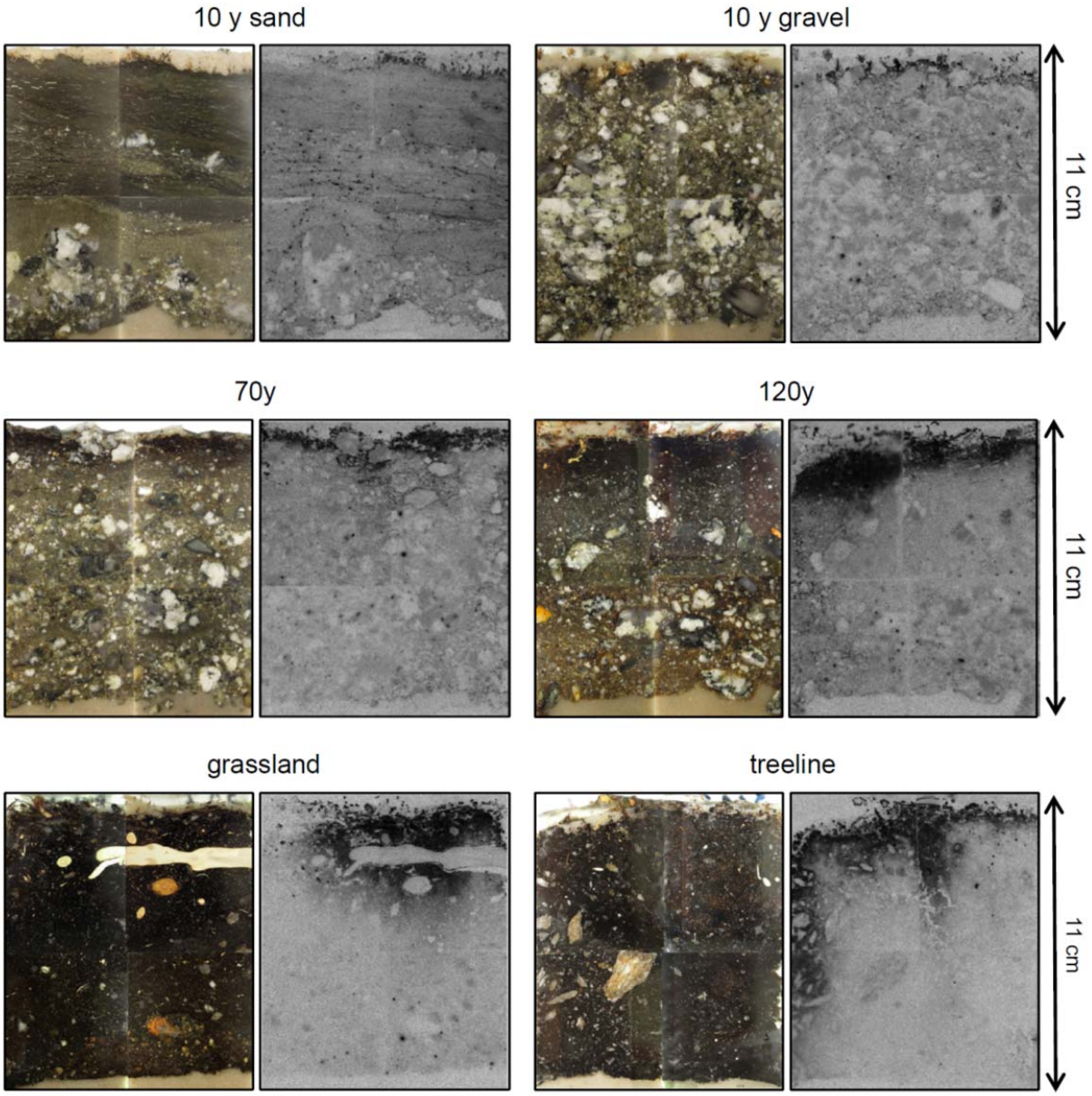

Fig. 4 Autoradiographic images showing the small-scale distribution of ^{14}C (darker pixels indicate higher labelling; right columns) in the soils of the chronosequence sampled 10 days after terminating the addition of litter-derived $DO^{14}C$. For comparison, the identical soil columns were photographed (left columns).

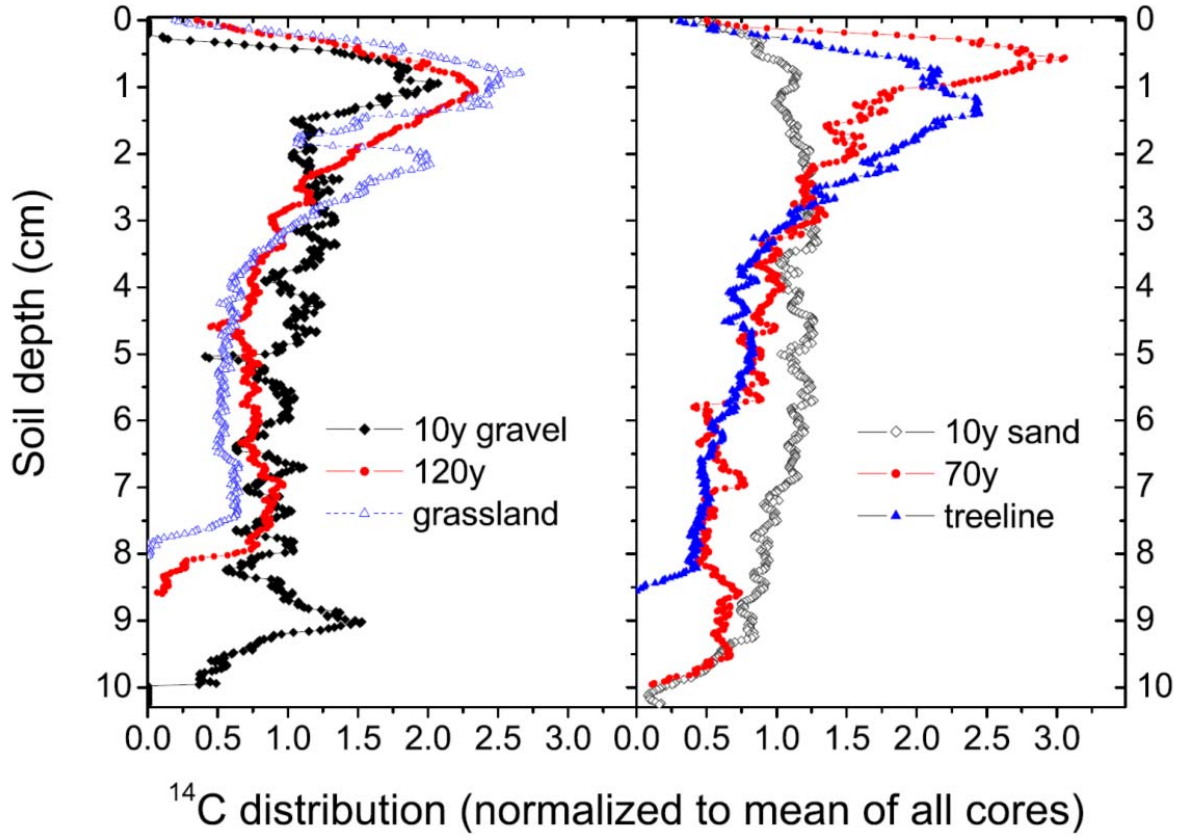

Fig. 5 Vertical distribution of ^{14}C activity at a 200 µm resolution estimated by autoradiography. Note that these data are standardized to the average label across all soil cores and that the soils had been sampled ten days after terminating the addition of litter-derived DO^{14}C.

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General Discussion

"Knowledge is infinite. The more you learn, the more you discover that you don't know anything."

- My grandfather -

In this thesis, I used ^{14}C labelling techniques to study effects of temperature and pedogenesis on C cycling processes. The results presented indicate that above- and belowground processes of the C cycle are tightly coupled and some of these are highly sensitive to temperature, especially under cold conditions.

The factorial manipulation of air and soil temperatures (Chapter 1) allowed me to show that most of the processes studied depend on soil temperature and these relations were mostly independent of air temperature. The soil temperature manipulations in the field (Chapter 2), demonstrated that soil temperature can determine net C uptake, belowground C transfer and use in the roots and the rhizosphere. Net C uptake increased steadily with soil temperature, while the transfer of recent assimilated to roots and rhizodeposition followed a non-linear relation, with a high temperature-sensitivity under cold conditions but negligible temperature effects at warmer conditions. Finally, the tracing of ^{14}C labelled DOC (Chapter 3) revealed that sorption at mineral surfaces is the most important mechanism of DOC retention and stabilization in soils, while preferential flow reduces DOC contact with the soil matrix, thus lessening its retention in the topsoil.

Temperature dependence of net C assimilation

The negative effect of low temperatures on net C assimilation (Chapter 1 and 2) can most likely be attributed to a cold limitation of C sink activities. Support for this mechanism is provided by previous studies, in which the authors observed an increase in foliar starch concentration in response to low soil temperatures, which is indicative of limited consumption or export of assimilates (Repo *et al.* 2004; Comerford *et al.* 2013). Although I did not analyze starch concentrations in my studies, the strong reduction of new assimilates allocation and respiration in the cold soil treatment provided evidence for reduced C sink activities in the roots. This could

have risen non-structural carbohydrate (NSC) concentrations in plant tissues, causing a downregulation of photosynthesis (Savitch, Gray & Huner 1997; Domisch *et al.* 2002).

Soil temperature effects on C assimilation were more pronounced in the field study (Chapter 2) than in the glasshouse experiment (Chapter 1), probably because of the dissimilar duration of the temperature treatment before plants were ^{14}C pulse labelled. There might be a certain time gap from the moment plants start experiencing C sink-limiting temperatures until NSC accumulation in the chloroplasts causes a downregulation of photosynthesis. Temperature effects on C uptake would therefore be stronger after 7 weeks (Chapter 2), than after 7 days (Chapter 1).

Alternatively, belowground transport, rather than consumption of new assimilates could have been hindered by low temperatures, causing NSC accumulation in the chloroplast and photosynthesis downregulation. Both assimilate transport and unloading from the phloem are temperature-sensitive. Phloem unloading relies on the enzymatic activity of sucrose invertases to hydrolyze sucrose to glucose and fructose (Sturm & Tang 1999) before entering other metabolic pathways. If the activity of these enzymes is inhibited by low temperatures, the flow of assimilates to the roots and other sink organs decreases.

Studies using C isotopes have also demonstrated the temperature-dependency of assimilate transport via the phloem (Hawkes *et al.* 2008; Plain *et al.* 2009; Mainali *et al.* 2014). Little is known, however, about temperature effects on sap flow under cold conditions. By studying root hydraulic conductivity in a 5–20°C temperature range, Wan *et al.* (2001) reported a steep increase of water viscosity and hydraulic resistance at low temperatures. The physiological girdling via phloem chilling performed by Johnsen *et al.* (2007) showed that the cooling of a relatively short section of trunk induces reductions in soil CO_2 efflux that equal those obtained with physical girdling by bark stripping. Consequently cold (albeit above 0°C) conditions seem to hinder belowground assimilate transport via the phloem.

These two mechanisms of cold-limitations of C transport and consumption are not mutually exclusive and could operate concomitantly.

The dependence of net C uptake on soil temperature implies that ground temperatures will drive plant productivity responses to warming in cold ecosystems. This is of high relevance because air and soil temperatures are going to be differently affected by global change. Changes in the snow regimes can have major effects on soil temperatures and consequently affect plant growth and phenology (Wipf & Rixen 2010). Snow manipulation studies have shown that later or smaller snowfall reduces winter soil temperature and increases soil freezing frequency with negative effects on plant growth (Groffman, Driscoll & Fahey 2001; Repo, Roitto & Sutinen 2011; Comerford *et al.* 2013; Rumpf *et al.* 2014). Given that soil temperature seems to drive C assimilation in cold ecosystems, attempts to predict ecosystem productivity under future scenarios should take into account the effect of soil temperature variation resulting from altered snow regimes.

The relation between soil temperature and C uptake could also explain the growth limitation of trees above the treeline (Körner 1998). As a tree cools the soil beneath while expanding its canopy, critically low temperatures induce assimilate accumulation, which inhibits C uptake and prevents the tree from further growing. Consequently, the trees scattered in this ecotone are characterized by a small-size and stunned shape.

Temperature dependence of belowground C allocation

By tracing ^{14}C labelled assimilates in roots and soils, I was able to quantify temperature effects on depth allocation and partitioning among the different plant and soil components. The investigation of interactive air and soil temperature effects (Chapter 1) revealed that the effect of soil temperature on belowground C partitioning can, in some cases, depend on air temperature,

while, by applying three equally spaced soil temperature treatments (Chapter 2), I found that belowground C allocation followed non-linear temperature relations.

For *P. mugo* belowground allocation of new assimilated C was higher in the treatment combination where air was warm and soil cold (Chapter 1). The mechanisms behind the interactive effect of air and soil temperature on belowground C transfer are not clear. Given the dependence of assimilate transport on trunk temperatures (Johnsen *et al.* 2007), it is possible that, in this combination, air temperature was not limiting the transport of new assimilated belowground, while decreased C consumption in the cold soil treatment caused an accumulation of new assimilates in the roots. Accordingly, autoradiographies presented in Chapter 1 revealed an interaction with air temperature on the belowground transport of new assimilates. In the cold air treatment, new assimilates were relatively more concentrated in the upper third of the pot, while more evenly distributed when air was warm. In agreement with our study Hoch (2013) observed an interdependency of growth response to temperature among above- and belowground organs, and suggested that plant hormones could play a role in linking above and belowground phenology.

Both autoradiographies and destructive analyses demonstrated that the belowground distribution of assimilates depends on soil temperature (Chapters 1 and 2), with less assimilates allocated to the deep soil when soils are cold. Such a relation could have resulted either from cold-inhibition of sink activities in the roots or cold-limitations on C transport into the deep soil. The experimental set-up I used did not allow disentangling these two potential mechanisms, but given that both assimilate transport and consumption are temperature dependent, both processes may have co-occurred.

The application of three equally spaced soil temperatures (Chapter 2) emphasized a non-linear temperature relation of assimilates transfer to soil, which was consistent among all the different C

pools investigated (microbial biomass, mycorrhizal hyphae and bulk soil) and was also reflected in respiration of new assimilates. Assimilate transfer to soil was strongly reduced in the cold soil treatment, while it did not differ between the two higher temperature levels. This pattern suggests that assimilate transfer to soil and respiration experiences a temperature threshold with a high sensitivity to temperature under cold conditions. Such relations could have important consequences for the response of C cycling to global warming. Accordingly to these relations, when temperature is not limiting belowground C allocation, warming-induced increases in plant biomass do not translate to increased soil C inputs via exudation and mycorrhiza. Consequently, warming alters C partitioning in favor of more persistent pools. On the contrary, under conditions where belowground C allocation and root metabolism are hindered by low temperatures, soil warming could strongly enhance the transfer of new assimilates to soil via root deposition. Quantification of soil C stocks in Tundra ecosystems under log-term warming provided support to this mechanism. Experimental warming enhanced root growth and exudates in the deep soil, which ultimately increased C stocks in the mineral horizon (Sistla *et al.* 2013).
Therefore, if warming in cold ecosystems enhances C inputs to the soil in form of root litter and exudates, the processes stabilizing these C compounds in the soil will determine whether this additional C input is going to be stabilized, decomposed, or lost with leaching.

DOC retention mechanisms and implications for warming in cold ecosystems

DOC retention mechanisms in soils have been investigated in Chapter 3. The tracking of ^{14}C labelled DOC revealed a small DOC biodegradation and fast immobilization of DOC in the upper soil, which correlated with the content of pedogenic oxides. We therefore concluded that sorptive retention on mineral surfaces is the dominating pathway of new DOC retention in soils. Sorptive

retention stabilizes DOC, reducing its susceptibility to microbial attack (Kalbitz *et al.* 2005; Scheel *et al.* 2008) and could therefore led to an increase of C stocks in the mineral horizon.
In our experiment we found little contribution of biodegradation to DOC removal from the soil. However, it is difficult to predict whether DOC biodegradation would differ in natural soils that contain living roots. Many studies have demonstrated that root exudates enhance microbial activity, thus stimulating the biodegradation of DOC (Kuzyakov, Friedel & Stahr 2000; Kuzyakov 2010). On the other hand, microbial activity is reduced at low temperatures. Microbial degradation of DOC could therefore be even lower in cold ecosystem than observed in our glasshouse study at 15-20°C.
I therefore conclude that, if enhanced root growth and activity cause and increase of C inputs to the soil in form of DOC, the retention and stabilization of this new C will depend on soil physio-chemical proprieties and water saturation. DOC stabilization will increase with the content of soil reactive mineral surfaces; nevertheless rising frequency of extreme meteorological events such as heavy rains and floods could hamper DOC retention, causing DOC to leach by preferential flow.

Temperature dependence of soil respiratory fluxes

In Chapter 1 and 2, respiration of new assimilates was more temperature-sensitive than total soil respiration. This observation challenges the common assumption that autotrophic respiration is less temperature-sensitive than heterotrophic respiration (Hartley *et al.* 2007; Wang *et al.* 2014). It further shows that temperature-relations strongly depend on the temperature range considered. Soil respiration of new assimilates presented the same temperature response of new C transfer to soil (Chapter 2), confirming that autotrophic respiration is strongly coupled to belowground C supply (Högberg *et al.* 2001). The allocation of assimilates to *P. mugo* roots, however increased steadily with temperature, reflecting increased assimilation rates, while the allocation of new C to

the soil was strongly suppressed in the cold treatment. Given that not only allocation to soil but also respiration of assimilates was strongly suppressed in the cold treatment, these results suggest that not the transport but rather the use of new assimilates in the roots and exudation were limited by low soil temperatures. Such a limitation could occur if phloem unloading is limited due to cold-inhibition of extracellular invertase activity (Canam, Unda & Mansfield 2008). These results go beyond the theory of a cold-limitation on root growth proposed by Körner (1998), suggesting that not only root growth, but also other metabolic pathways are inhibited low temperatures, thus constraining new C transfer to soil via exudation and mycorrhizal associations. Soil warming in cold ecosystems would therefore not only enhance root growth, but also C transfer to the soil via root exudates and mycorrhizal associations, which could lead to an increase of soil C stocks.

Outlook

Although limited to two species and only few temperature levels, my analyses suggest that the effects of warming on C partitioning will depend on the initial conditions under which warming occurs. If belowground C transport is not limited by low temperatures, warming increases partitioning aboveground with relatively small or no change in rhizodeposition. As such, warming would increase C partitioning to more stable plant pools (biomass) and reduce C inputs into soil organic matter. On the contrary, if warming occurs under cold-limiting conditions, root respiration and rhizodeposition are strongly enhanced, increasing C partitioning to more rapidly cycling pools. In relative terms, a larger part of the C allocated belowground will be either used to support root metabolism or be exuded to the soil and, if not stabilized, decomposed.

It should be acknowledged that the interpretation of the results here reported is restricted by the short duration of the treatment and the relative simplicity of the system studied. For instance, this study did not take into account increasing soil C inputs via litter deposition, which could

consistently increase soil C stocks (Qian, Joseph & Zeng 2010; Sistla *et al.* 2013). Furthermore, plant interactions, changes in community compositions, acclimation processes and reduction of liable soil C stocks may cause long-term responses to substantially differ from short-term observations. Nevertheless, temperatures are very dynamic throughout a growing season, and understanding short-term C allocation responses thus crucial to accurately estimate the seasonal patterns of C cycling. Also, the frequency of extreme meteorological events is projected to increase in the future (IPCC, 2013), underlining the need to understand responses of the C cycle to both short- and long-term temperature changes.

In this thesis, I have focused only one aspect of climate change, warming. While I believe that gaining insight into temperature effects on C cycling processes can consistently improve our understanding of ecosystem's response to warming, it is also important to acknowledge that such response depends on many other drivers and on their interactions. Increased nutrient availability, changes in soil moisture and higher CO_2 concentration are other key aspects of global change affecting plant growth and decomposition. Because (with exception of CO_2 concentrations) alterations of these drivers are heterogeneous in space and time, it is important to assess their combined effects.

Conclusions

This thesis provides new insights into the temperature relations of C cycling processes and mechanisms stabilizing plant derived C in soils. The work presented here shows that cold soils inhibit net C uptake. I have attributed this inhibition to the downregulation of photosynthesis induced by product accumulation. Accumulation of assimilates could have resulted from cold-induced limitations on metabolic, enzymatic and physical processes. More specifically, low temperature may have reduced root metabolisms and thus the consumption of assimilates, or

inhibited the enzymes responsible for the unloading of assimilates in the phloem. Physical constrains could also limit assimilate transport to the sink organs, if sap flow is reduced because of increased viscosity.

I have further shown that not only root growth, but also C transfer to soil via rhizodeposition and mycorrhizal associations are strongly suppressed in cold conditions. Warming will consequently alter C partitioning, increasing C inputs to the soil via rhizodeposition. The soil content of reactive mineral surfaces is going to determine whether these additional inputs will be stabilized or quickly biodegraded.

My work also underlines the existence of non-linear temperature relations, as belowground C allocation and respiration were strongly temperature-sensitive under cold but not warm conditions. I conclude that soil warming will strongly affect C cycling processes in cold ecosystems and believe that air and soil temperature, and their temporal variation and covariation, need to be considered to accurately to forecast C cycling under future climatic conditions.

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"Some people come in your life as a blessing. Some come in your life as a lesson."

- Mother Theresa -

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Curriculum Vitae

"Be the change you wish to see in the world."

- Mahatma Gandhi -

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Publications

Adele Ferrari, Frank Hagedorn, Pascal A. Niklaus
Experimental soil warming and cooling alters the partitioning of recent assimilates: evidence from a 14C-labelling study at the alpine treeline
Oecologia (First online: 28 August 2015)

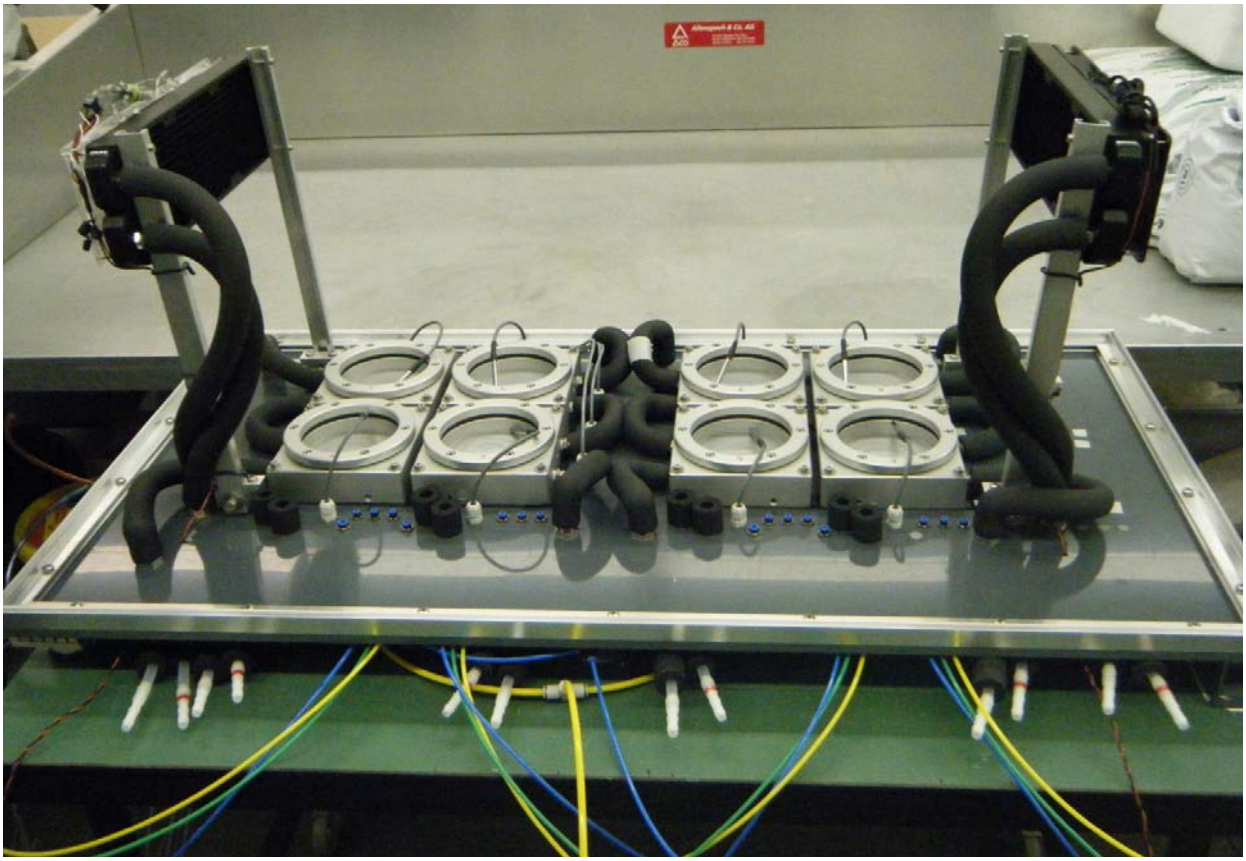
Frank Hagedorn, Nadia Bruderhofer, **Adele Ferrari**, Pascal A. Niklaus
Tracking litter-derived dissolved organic matter along a soil chronosequence using ^{14}C imaging: biodegradation, physico-chemical retention or preferential flow?
Soil Biology and Biochemistry (88), 333-343, 2015

Lisa Eggenschwiler, Maya Senn, **Adele Ferrari**, Andreas Egli und Katja Jacot
Attraktivität von extensiven Wiesen für Blattlausfeinde
Agrarforschung Schweiz 3 (2): 96–103, 2012

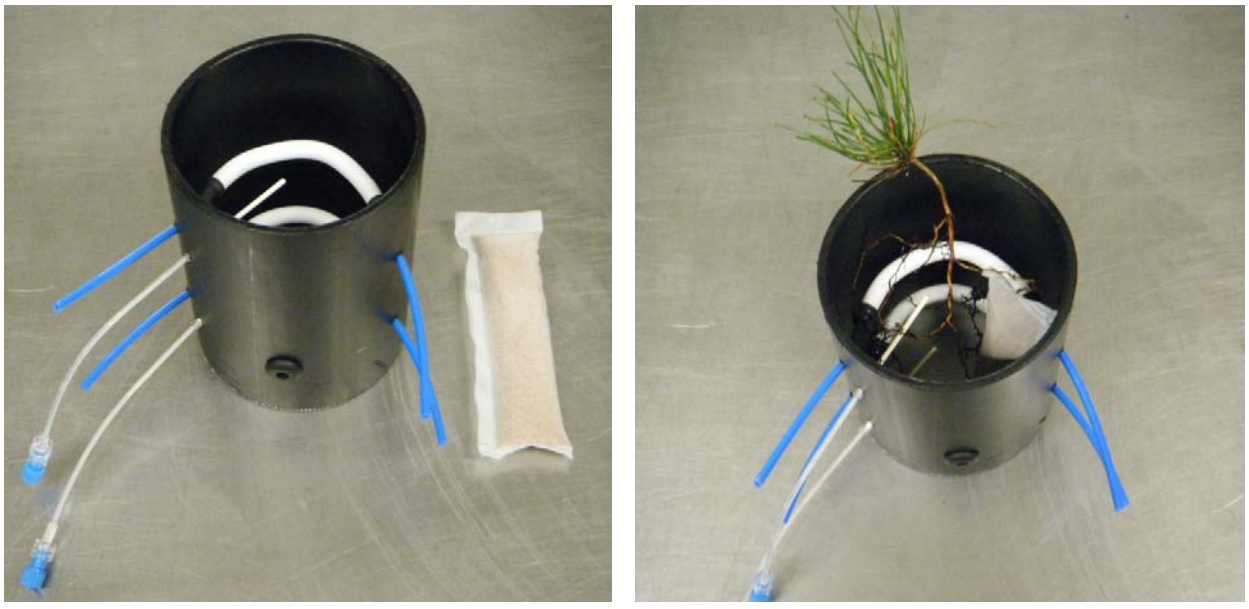
Manuscripts in preparation

Adele Ferrari, Frank Hagedorn, Pascal A. Niklaus
Interactive effects of air and soil temperature on C allocation in cold-adapted plant species: A ^{14}C pulse labelling experiment
(in submission)

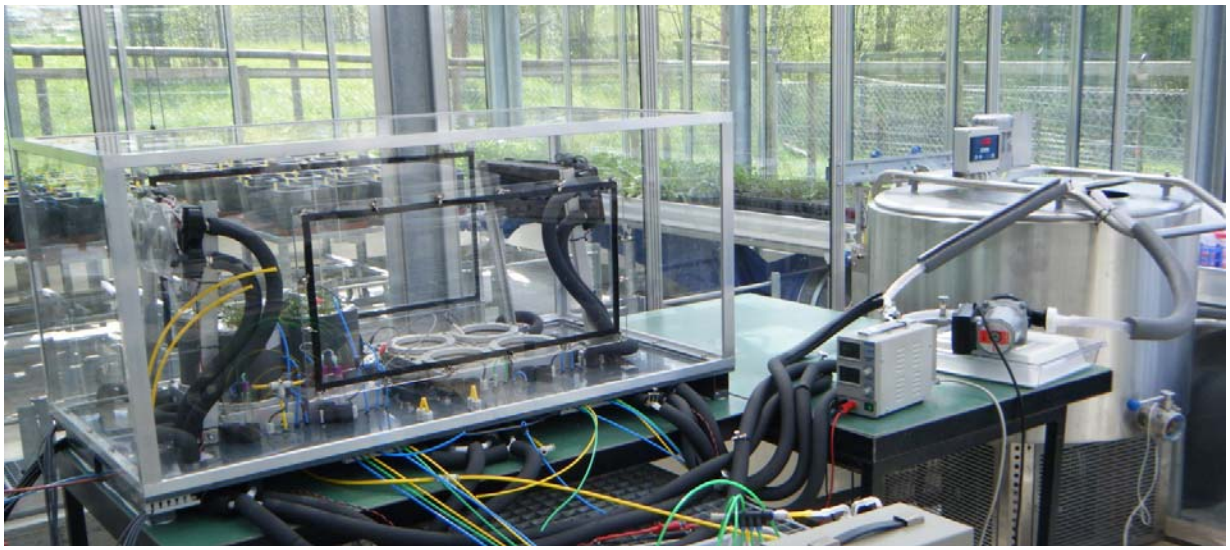
Annexes



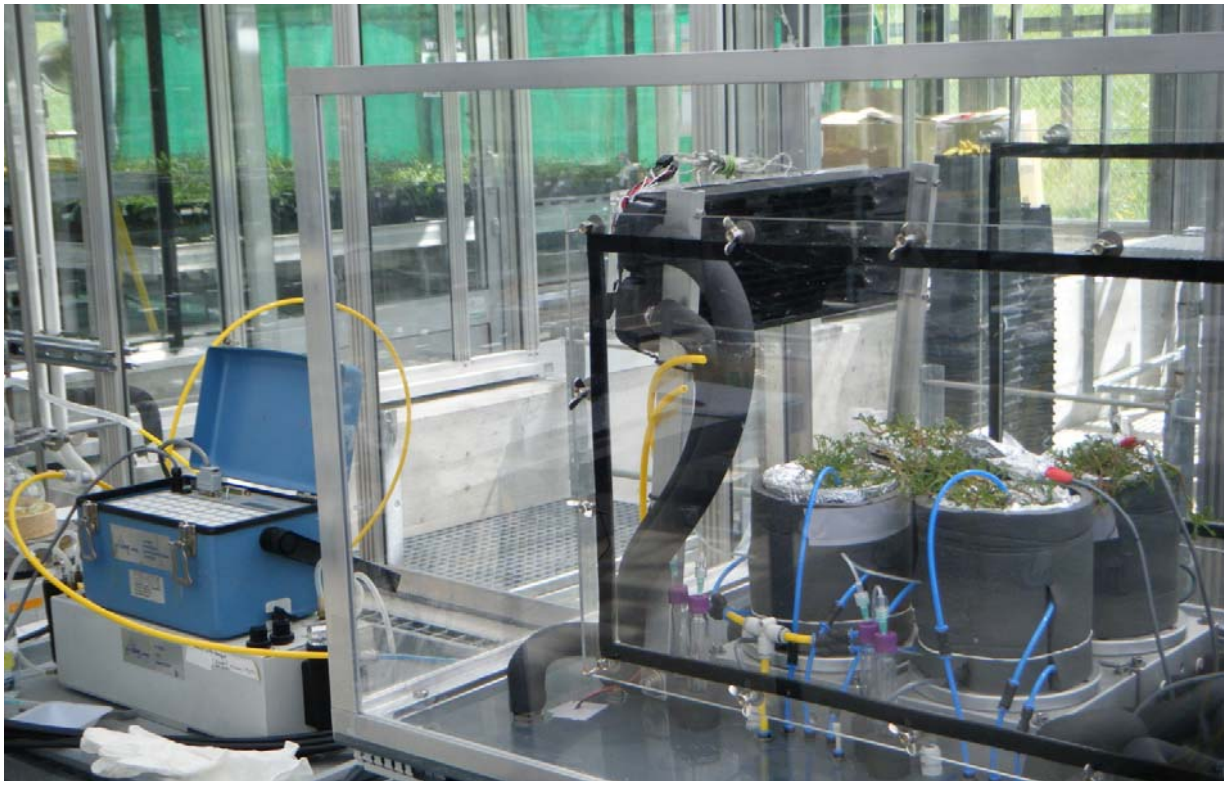
Chapter 1: Air and soil temperature controlling system



Chapter 1: Experimental pot with rhizosamplers, accurel tubing and sand bag



Chapter 1: Labelling chamber and cooling bath



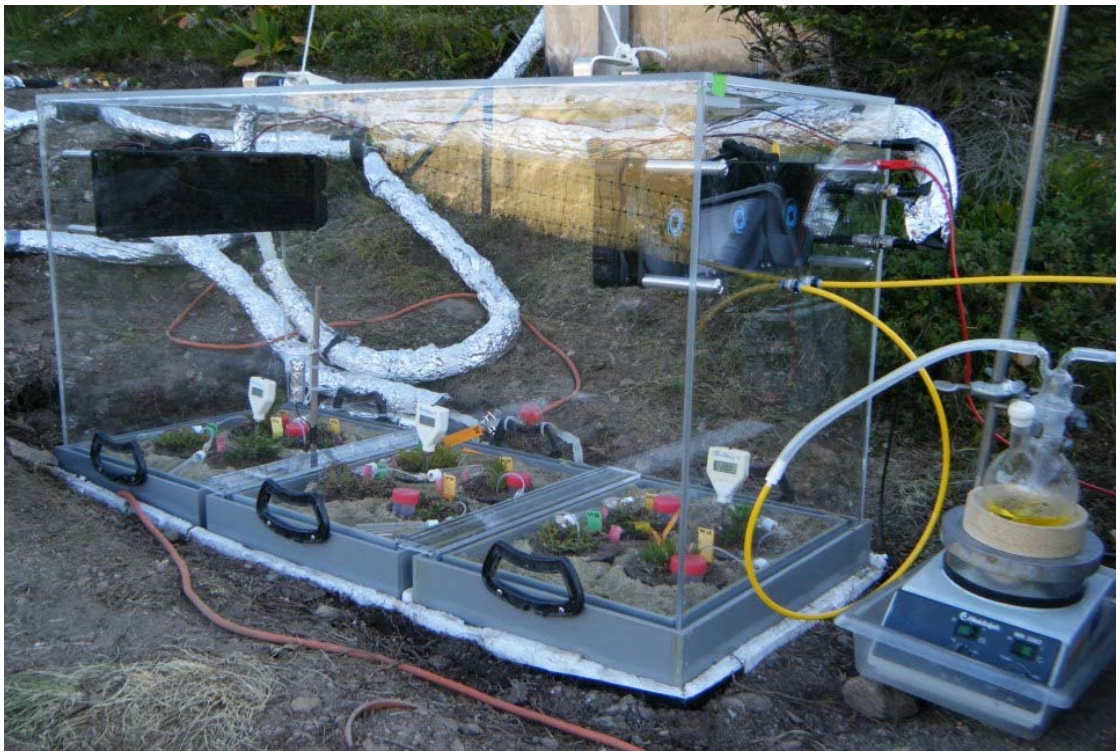
Chapter 1: Experimental pots with cell foam insulation, soil respiration and DOC collecting system

Chapter 2: Insulation between the plots

Chapter 2: *P. mugo* and *L. alpina*

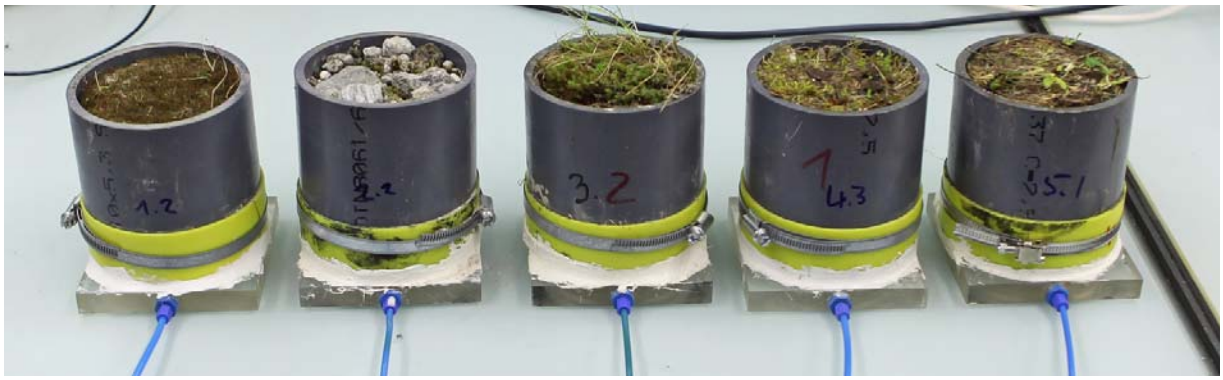
Chapter 2: Soil respiration and DOC collection

Chapter 2: Mini-respiration chambers and DOC collection

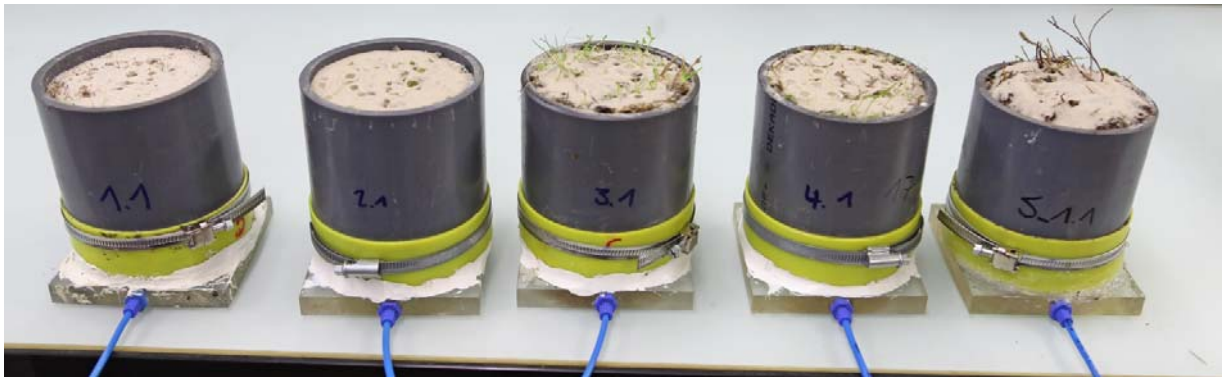


Chapter 2: ^{14}C labelling chamber

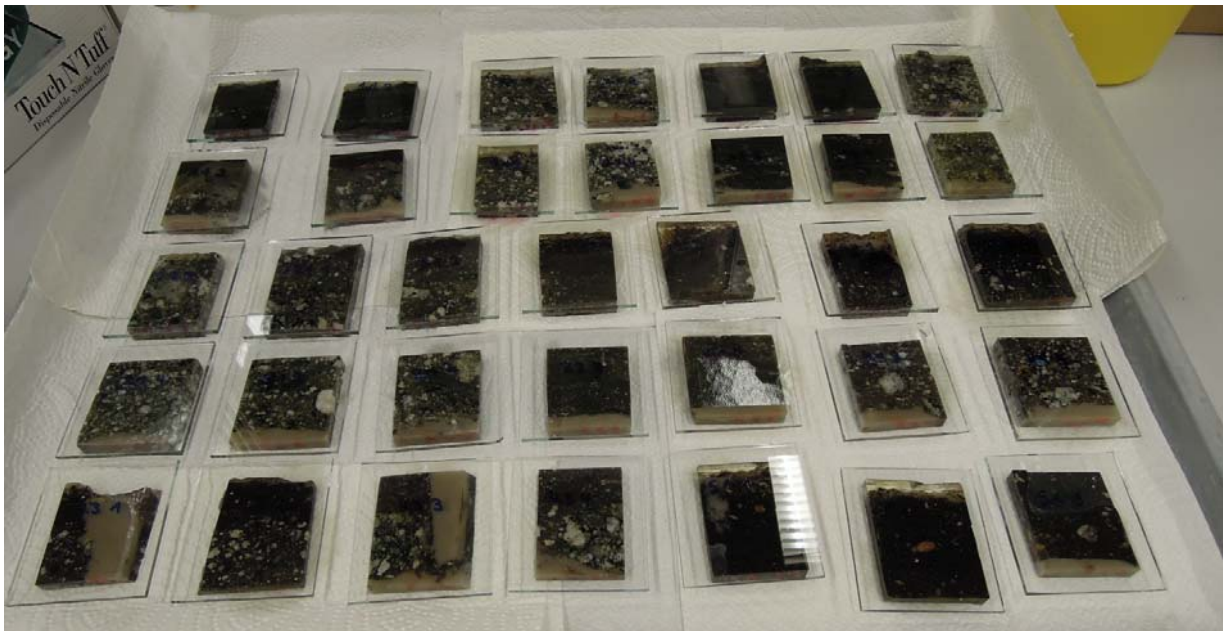
Chapter 2: Experimental setup with the structure to lift the labelling chamber



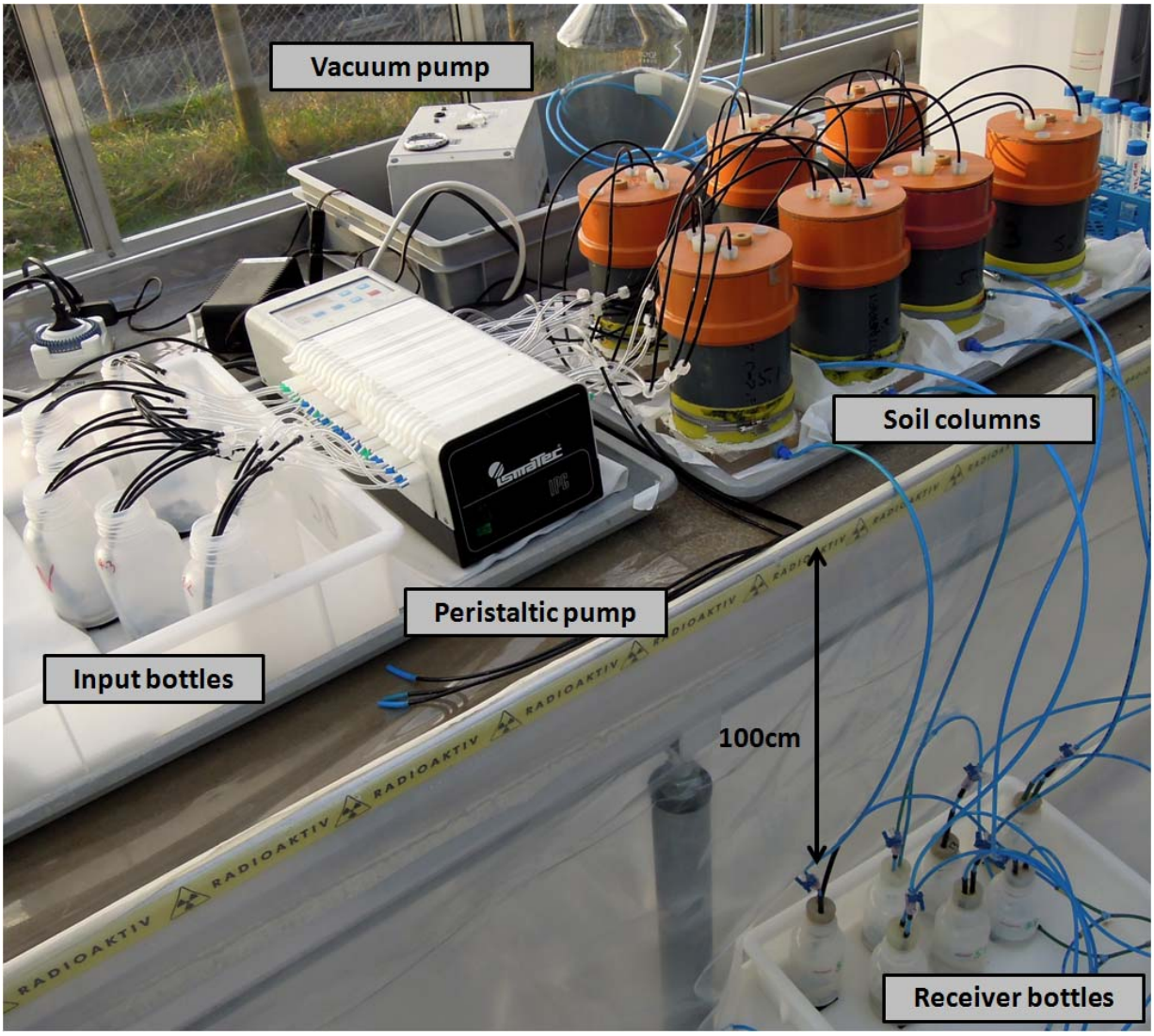
Chapter 3: Soil columns fixed on the porous borosilicate plates



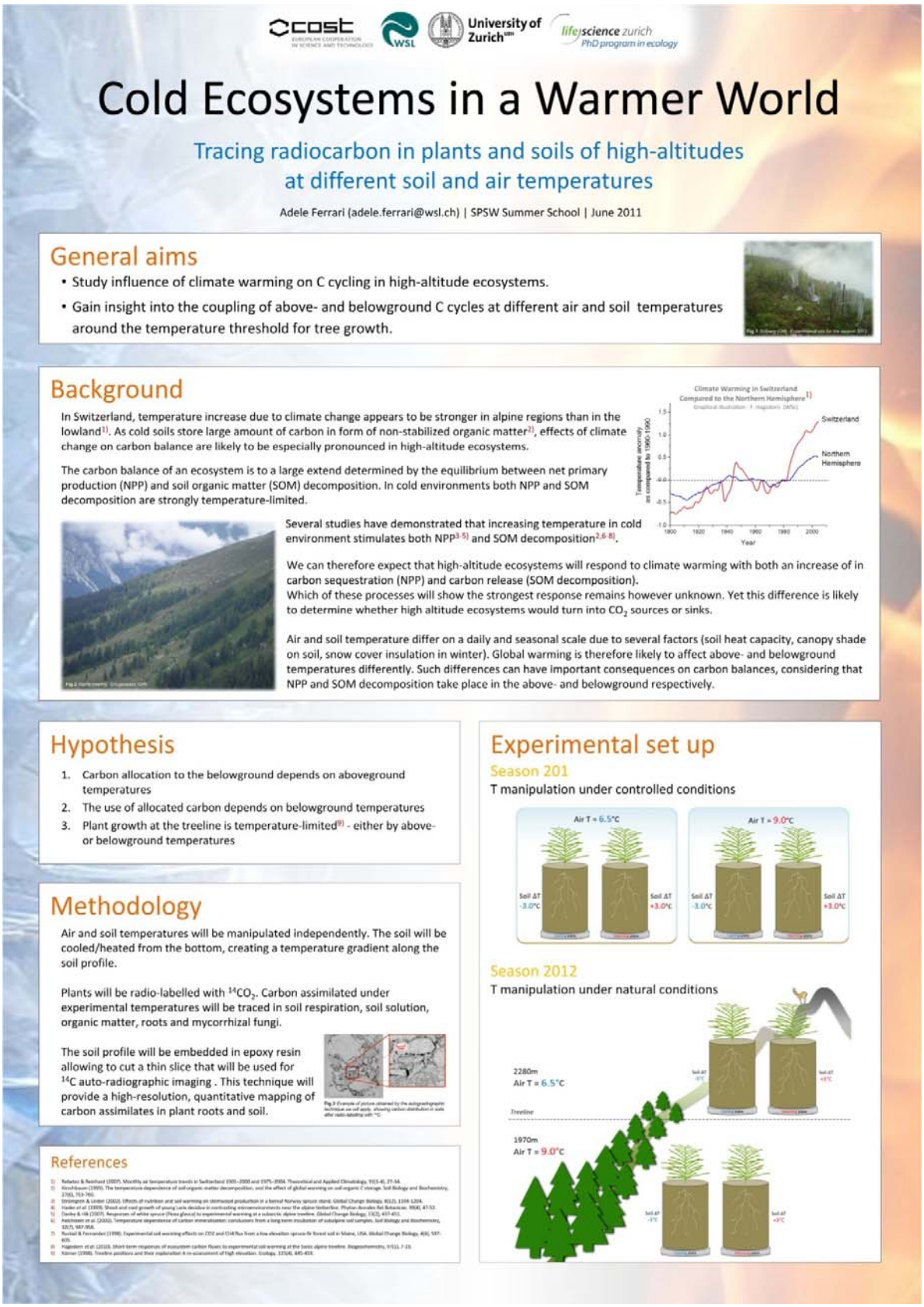
Chapter 3: soil columns covered with a layer of quartz sand preventing splash erosion while addinng the $DO^{14}C$ solution



Chapter 3: Resinified soil slices used for autoradiographic imaging



Chapter 3: Experimental setup



Poster presented at the SPSW Summer School "Terrestrial Ecosystem Dynamics in a Changing World", Mürren (CH) 2011. Received award for the **best poster**

Cold Ecosystems in a Warmer World

Tracing radiocarbon in plants and soils of high altitudes at different soil and air temperatures

Adele Ferrari, Frank Hagedorn, Pascal Niklaus

Motivation

Alpine treelines are characterized by a sharp shift from upright growing trees to low status alpine plants. The mechanisms for the abrupt growth form change are not well understood. As treelines occur at mean soil temperatures of about 6°C during the growing season, low temperature seems to be the principal factor limiting tree growth. It remains, however, unclear whether air or soil temperature are growth limiting, and how belowground C allocation responds to temperature changes.

Hypotheses:

- Low soil T limits belowground C allocation and above-ground growth
- T sensitivities of C uptake and belowground C allocation differ

Fig. 1 Experimental site at Stillberg near Davos at 2280 m a.s.l. just above the "natural" treeline.

Experimental set-up

Study site
Stillberg in the Swiss Alps, 2280 m a.s.l.

Plant species
Pinus mugo
Leucanthemopsis alpina

Soil temperature manipulation
ambient (control), warming and cooling by ±6.5°C

Warming was performed with heating cables; cooling with anti-freeze solution precooled to -2.5°. Both were buried at 15 cm soil depth resulting in temperature gradients towards the soil surface.

Fig. 2 The six experimental blocks when the soil temperature manipulation began

Fig. 3 Air tight plexyglass chamber used for the radio-labelling

Warming or cooling soils from June to mid August 2012 resulted in average soil temperatures at 3 to 10 cm depth of 5.9, 12.7 and 19.2°C.

^{14}C labelling

In August, all plants were block-wise labelled with $^{14}CO_2$. Soil $^{14}CO_2$ efflux was trapped in small soil respiration chambers containing 2 ml of 1M NaOH (Fig. 5).

^{14}C was quantified in trapped CO_2, plant tissue, soil microbial biomass and soil organic matter, sampled 5 days after radio-labelling by liquid scintillation counting.

Fig. 5 Mini-soil respiration chamber

Results

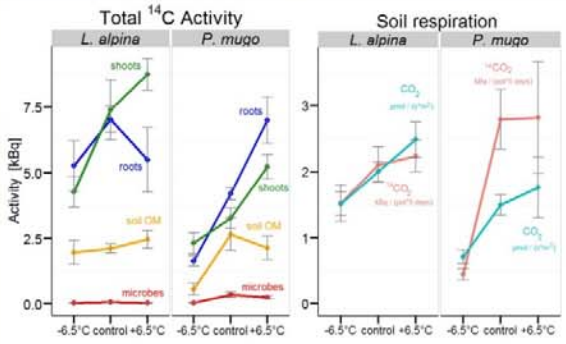


Fig. 6 Averaged ^{14}C activity in *L. alpina* and *P. mugo* total plant biomass, soil organic matter and microbial biomass (n=6 blocks). Activity is reported as the total for a plant-soil unit (total plant and soil material present in one pot).

Fig. 7 Comparison of CO_2 and $^{14}CO_2$ in soil respiration, measured in pots with *L. alpina* and *P. mugo* plants during 5 consecutive days following pulse labelling (n=6 blocks).

- ^{14}C in above-ground biomass correlated positively with soil temperature for both species. In roots, ^{14}C was lower in cooled soils with *P. mugo*, but not in *L. alpina* (Fig. 6).
- Total soil respiration increased linearly with soil temperature.
- Soil-respired $^{14}CO_2$ did not differ between warmed and control treatments, but much less $^{14}CO_2$ was respired in the cooled treatment (Fig. 7). The same pattern was observed with *P. mugo* for ^{14}C in microbial biomass and soil organic matter (Fig. 6). Our results indicate the existence of a temperature threshold for a C metabolization below ground.

Conclusions

- Low soil temperature restricts above-ground growth
- C allocation and metabolization of recent assimilates in the rhizosphere becomes limited by low soil T, particularly for *P. mugo*.
- Components of soil respiration respond differently to soil warming: SOM decomposition increases linearly with T, while rhizosphere respiration shows a threshold-driven response.

Poster presented at the PSC Summer School "Green Revolution Reloaded: Emerging Technologies for Sustainable Crop Production", Einsiedeln (CH) 2014. Received award for the **best poster presented**